

VERTEBRATE NEUROSECRETION

A REVIEW

L S RAMASWAMI, FNA



**INDIAN NATIONAL SCIENCE ACADEMY
Bahadur Shah Zafar Marg, New Delhi-110002**

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INTRODUCTION

The study of neurosecretion had its inception in the early part of the 20th century. That highly specialised nerve cells or neurons could also function as glandular ones was a major breakthrough. The secretion arising from a localised part of the Central Nervous System called the hypothalamus entered the general body circulation; it also reached that part of the pituitary gland called the adenohypophysis through a portal circulation and triggered secretion of pituitary tropic hormones. Maintenance of internal homeostasis was largely due to these chemical messengers. The science of Neuroendocrinology is new but has already gathered vast literature and it has been a herculean task to collect, sort and judiciously include the relevant ones under 'References' in writing up this review. Authoritative Schools have sprung up in different parts of the world in reproductive biology and human fertility control using neuroendocrinological knowhow. For example, to prevent conception, it may be possible to time almost exactly ovulation in the human female by using synthetic LRH. In developing countries where population explosion is bedevilling economic growth, more and more students should devote to the study of Neurosecretion in its various profiles which may help solve the above vexed problem. Towards giving a comprehensive backdrop to students desirous of studying Neurosecretion, I have put my ideas on paper, having taught the subject over a number of years. In doing this, I am fully conscious that the essay is not the last word in the subject nor does it aim to be a highly critical one as experts would want it. My only hope is that the student world to whom it is primarily addressed, consisting of not merely biologists but others as well, will find it useful in their pursuit of reproductive biology and comparative endocrinology. Researchers could also use it as the information is fairly updated. In all these, I am always remembering that as soon as a tome comes out of the press, its half-life is getting to be over!

Vertebrate Neurosecretion—Review

L S RAMASWAMI, FNA*

(Received on 16 April 1979)

The Relation between the Hypothalamus and Hypophysis

Dahlgren (1914) and Speidel (1919) described in the spinal cord of sharks certain glandular cells; this marked the beginning of the study of vertebrate neurosecretion. In the late 1920s, Ernst Scharrer (1928) and later Sloper (1966) described certain glandular nerve cells from the preoptic nucleus of the hypothalamus of the minnow (*Phoxinus laevis*) and speculated upon their endocrine functions. In the late 1940s, it was placed on a firm footing when the axons of these cells were found ending up in the neurohypophysis. While the hypothalamus, a part of the brain is primarily concerned with the functions like heat, sleep, appetite regulation etc., by neural mechanism, it also monitors hormonal secretion of the hypophysis. The present concept is a hypothalamo-hypophysial system. Thus the hypothalamus was recognized as an independent endocrine gland.

It was for a long time thought that the pituitary gland (hypophysis) (PG) was a master gland controlling the activities of a number of endocrine glands. It is now known that there is another part of the

central nervous system (CNS)—the hypothalamus referred to above, which has profound influence on the hypophysis so that the emphasis has shifted to the hypothalamus to the hypophysis; the neuron Neural information passes from the hypothalamus to the hypophysis; the neuron functions like a transducer. If the hypophysis or pituitary gland is removed from its site and transplanted elsewhere (eyemuscle/under kidney capsule) (Martini et al. 1959, Jørgensen 1970) production of tropic hormones falls dramatically in the heterotopic transplant, as e.g., in the Amphibia, adrenocorticotrophic (corticotrophic) hormone, thyroid-stimulating hormone, and gonadotropins first reach zero level and then rise to normal in the adeno-hypophysis; prolactin and intermedin (which are under the inhibitory control of the hypothalamus) are secreted in large quantities (Gorbman & Bern) 1962). The hypothalamus is unable to regulate other hormone secretions (Martini et al. 1959). If, however, the hypophysis that has apparently lost its function is retransplanted back and the blood connexions are re-

*Present Address: 387, Upper Orchards, Bangalore 560080, India.

established, it regains its physiological functions in course of time. The same results could be obtained if a cellophane piece is intercepted between the hypothalamus and the hypophysis, thereby disrupting the vascular continuity, or even a cautery of the median eminence would do the trick. Holmes et al. (1959) experimented this by introducing a polythene film between the cut end of the stalk in monkeys so that the pituitary gland was severed of the hypothalamic control. They found that the adrenal and thyroid functions were not affected but there was a loss of ovarian cyclical activity. Even if the vascular connexions between the hypothalamus and the pituitary were re-established, the gonadotropic activity was not resumed. However, in spite of the foregoing, a conclusion is inescapable that the vascular connexion plays an important role and that the hypothalamus secretes a substance (hypophysiotropin, chaemotransmitter, neurohormone, neurotransmitter) which controls the secretion and release or inhibition of the hypophysial hormones in a masterly way.

If this is accepted, then what is the relationship between the hypothalamus and the hypophysis? While the posterior pituitary (neural lobe) receives a large number of neurosecretory axons (neuraxons), the anterior lobe of the pituitary receives none; or it may be scanty innervation (McCann & Dhariwal 1966). Harris (1948) called it "a gland under nervous control but lacking nerve supply". A few vasomotor fibres do enter the pars distalis or anterior lobe but these go to the walls of the blood vessels (Greep 1963). As early as 1966, Gabe mentioned that in the sea-horse (*Hippocampus guttulatus*), the axons from the hypothalamic region end in close contact with the acidophil and basophil (gonadotropic) cells of the mesoadenohypophysis, a feature also noticed in a few

other teleosts. Then how does the anterior lobe function? In the tetrapods, viz., frogs, reptiles, birds and mammals (and quite a few fish) there is a system of blood vessels connecting the median eminence (ME) part of the hypothalamus with the anterior lobe (pars distalis or glandularis)—the portal system (figure 1). The median eminence is irrigated by the hypophysial artery on either side coming from the internal carotid artery and forms the primary plexus there. The venous capillary network arising in the median eminence, forms the portal veins; the latter enter the pars distalis (PD) and form the secondary plexus bathing all the secretory cells there.

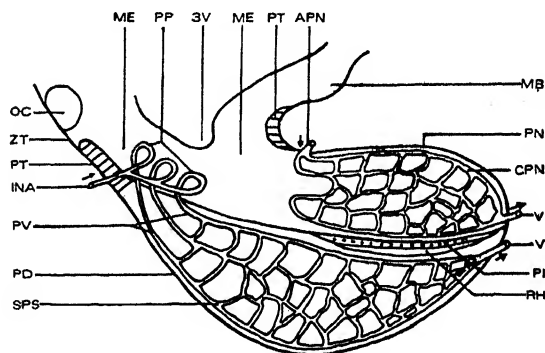


Figure 1 Anatomy of the hypothalamus and hypophysis of a mammal showing vascularization of the pars nervosa and the portal circulation in the pars distalis. Note there is no separate arterial supply to the pars distalis

This may be examined in greater detail as described by Holmes (1967) in a mammal. The superior hypophysial artery (figure 2) entering the hypothalamus forms the subependymal capillary plexuses. From these, loops arise in the region of the ME and these constitute the primary plexus. The capillary plexus referred to above and the loops are connected by a longitudinal vessel. In higher mammals (man), in addition to loops, a system of complex spikes

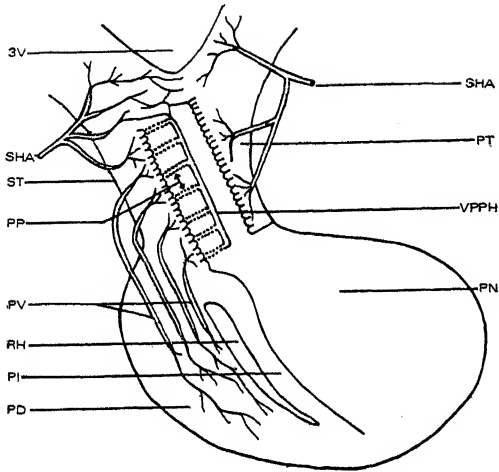


Figure 2 The portal circulation of a mammalian hypothalamus and pituitary gland based on an ink-injected specimen (After Holmes 1967, modified)

(described so as they resemble botanical formations) are also formed (see Daniel 1966). From these loops arise vessels which form the portal veins; some of these superficial ones pass through the pars tuberalis (PT) into the pars distalis and break up into sinusoids. Holmes supported a zonal distribution of portal blood. He also said that there was a morphological possibility of flow of blood directed towards the hypothalamus in many species. Flament-Durand (1977) feels that selective neurons synthesizing selectively hormones is not yet confirmed. While the link, therefore, between the supraoptico-paraventricular nuclei (figure 3) and the posterior lobe is purely neural, that between the parvocellular neurons and the pars distalis is neurovascular mediated by the portal system of the ME which, therefore, is a neurohaemal organ of the vertebrate neurosecretory system.

The Portal System

The hypothalamo-hypophysial portal system as described above in the majority of

vertebrates was discovered by Popa and Fielding (1930) and they described the direction of blood from the pituitary into the hypothalamus. Later, Wislocki (1937) described the then current correct direction of blood flow from the ME into the pituitary gland. Obviously the activity of the pituitary gland is controlled by the neurosecretory substance or neurohormone produced by the hypothalamus (the actual site of the synthesis of these hormones will be described later) and this has been known since 1955. The presence of these neurotransmitter substances has been assayed in the portal blood. These substances, when injected, are found to release specific hormones from the pituitary gland. Peculiarly, in frogs while most of the portal capillaries enter the pars distalis, a few pass into the parenchyma of the pars intermedia (PI).

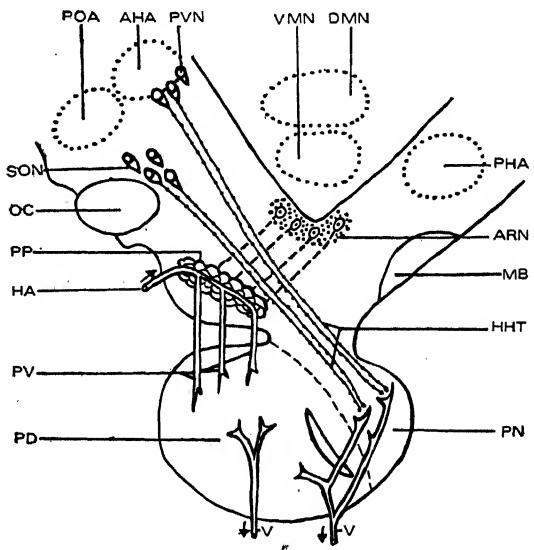


Figure 3 The portal circulation and the magnocellular and parvocellular neurosecretory tracts of man, diagrammatic. Note a number of arcuate nucleus neurons sending axons to the primary plexus (After Flament-Durand 1977, modified)

While the majority of authors (Gorbman & Bern 1962, Nalbandov 1970) described a flow of blood from the hypothalamus to the pars anterior (PA), Török (1962) described a flow in the opposite direction also in the dog. According to the Hungarian School (to which Török belongs), some blood from the posterior surface of the PA passes towards the posterior lobe vascular system. The latter may also be drained towards the hypothalamus (Szentagothai et al. 1968, p. 90). According to this School, the chromophobes synthesize argyrophil Bodian positive granules which pass into the hypothalamus (median eminence) by a backflow of the blood referred to above (see figure 46, and Szentagothai et al. 1968), and this 'internal' feedback mechanism is probably related to the adrenocorticotrophic function.

Porter et al. (1974) after conducting a series of experiments on portal circulation came to the conclusion that the observation of Török of a back-flow in the portal vessels into the hypothalamus may be due to an artefact observed normally in a dead or nearly dying animal.

Be that as it may, it has been brought out again (Sétáló et al. 1978) that the small side branches of the ME capillary loops in the cat and dog could be traced to reach below the third ventricular ependyma; in these the blood flows in the opposite direction, i.e., from the ME into the medial capillary network of the hypothalamus. The further studies of the Hungarian School brought out that in the rat the same internal vascular plexus has similar connexions, i.e., with the primary plexus in the PT and with the mesial capillary network around the lower part of the 3rd ventricle. In the injected living animals it was noticed that blood coursed in the internal vascular plexus towards the subependymal capillary network of the hypothalamus. According to these authors

there is the portal system irrigating the PD; the portal vessels may arise directly from the plexus of the PT and also from the ME capillary loops which also arise from the PT plexus. In addition to the blood passing from the PT into ME and then through the portal vessels into the PD, they described three other routes:

1. a small quantity of blood after coursing through the PT and then the loops of the ME, may be drained through the interior vascular plexus of the ME towards the subependymal (medial) capillary network of the hypothalamus,
2. the blood reaching the posterior surface of the PD sinuses courses upwards and is drained towards the pars nervosa vascular system between the PD and PI,
3. some blood from route 2 described above enters the internal vascular plexus of the infundibular stem and finally goes to the hypothalamus.

More work (Page et al. 1978) done extensively using scanning EM on the rat, rabbit, sheep, dog, cat and monkey has brought out many interesting points and some supporting the Hungarian School. According to them the capillary network of the ME is in two parts: an external [corresponding to the superficial of Duvernoy (1972)] and an internal (the deep plexus of Duvernoy) plexus; the internal plexus is a continuation of the external. A ring of superior hypophysial arteries arising from the circle of Willis encircles it; the arterial supply is restricted to the external plexus. With regard to the hormonal contents of the external plexus and the portal blood, the latter shows the hypothalamic factors, neurohypophysial and adenohypophysial hormones. In the rat, if the posterior

lobe is selectively ablated, melanocyte stimulating hormone (MSH), prolactin and Adrenocorticotrophic Hormone (ACTH) levels fall in the portal blood and this leads one to infer that the adeno-hypophysial blood gains access into the ME through the neurohypophysial capillary bed.

The blood in the ME rich in adeno-hypophysial, hypothalamic and neurohypophysial factors may flow in three ways:

1. to the adeno-hypophysis by capillaries directly or by fenestrated portal vessels,
2. to the medial basilar hypothalamus by direct capillary connexions,
3. to the internal plexus of the ME and thence to the adeno-hypophysis.

In their study of the rabbit pituitary angioarchitecture, Page et al. (1976) have observed that the arterial supply is only to the neurohypophysis. In the ME, there is an external and internal plexus and the blood supply of the latter is derived from the former before it opens into the adeno-hypophysis. It is also pointed out that the *entire* neurohypophysis not only serves as a pathway to the PD but also to the releasing and inhibiting, and posterior lobe hormones to the cerebro-spinal fluid (CSF) which may ultimately reach the brain. They do not refer to the PT or its plexus.

In the monkey (Page et al. 1978), the angioarchitecture of the ME internal plexus (figure 4) is divided into four zones: (1) the simple capillary loops (long and short) arise from the ME and the infundibular stem; these probably have no separate arterial supply; (2) the 'tufted vessels' are infoldings of the external plexus and open into a core vessel which goes to the PD; (3) the capillary spikes of the posterior tuber also arise from the external plexus and join the hypothalamic capillaries; (4)

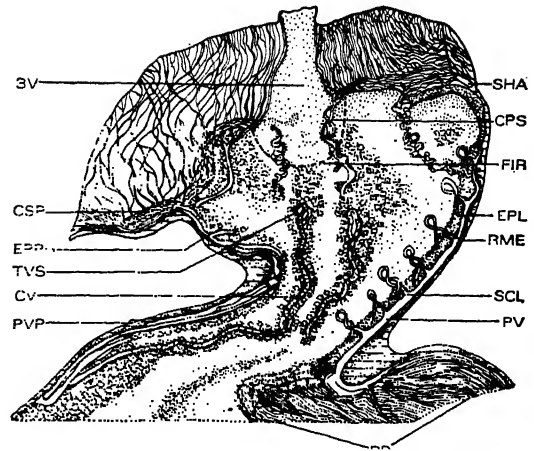


Figure 4 Angioarchitecture of the monkey medial basilar hypothalamus, median eminence and pituitary—a mid-sagittal reconstruction (After Page et al. 1978, modified). Note that some pituitary hormones may be carried to the hypothalamus also

the capillary skeins also arise from the external plexus and become associated with the floor of the 3rd ventricle where tanycyte ependyma are present. It is not clear whether the numerous hormones (neurophysin in the 3rd ventricular cavity of the monkey, LHRH and TRH in the rat 3rd ventricular fluid, ADH in the tanycyte ependyma of the rat ME) have been transported by the tanycyte ependyma, but structurally the ME ependyma is ideally suited for this. They state that "As the study of the median eminence angioarchitecture led Wislocki and King ('36) and Green and Harris ('47) to the concept that neurosecretion to the adeno-hypophysis regulates the anterior pituitary function, so further study of pituitary angioarchitecture has led us to the concept that pituitary secretion to the brain occurs and will be found to modulate cerebral function".

In the toad, Rodriguez and Piezzi (1967) described an encephalo-posthypophysial portal system (figure 5) which receives

three primary plexuses: (1) from the region of the preoptic nucleus, (2) from the anterior and (3) posterior regions of the mesencephalon. Such a system with its primary plexus in the hypothalamus and mesencephalon, forming a secondary plexus in the PI is seen in anurans (Cruz 1959); there is no arterial supply to the PI in view of its portal supply. Hasegawa (1969); gives a figure of the encephalo-posthypophysial portal system of *Bufo vulgaris* which I have also reproduced (figure 6) with a little modification. It may be noted that there are separate arterial supplies to the PD and PI. There are three sets of portal veins called first, second and third by him. The vein running between the PD and PI shown by Rodriguez and Piezzi (1967) is not described by Hasegawa.

An encephalo-neurohypophysial portal system is absent in Amniota.

Pars tuberalis: It is interesting to note that in Anura, the PT is situated far anteriorly to the PD and quite separately from it. It has a vascular supply but no portal system in the mammals investigated (Flerko 1966). It shows PAS positivity (and therefore secretory), presence of tropic hormones and occasionally mitotic activity. It

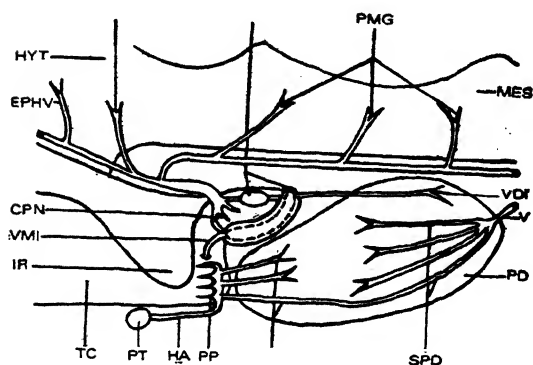


Figure 5 Encephalo-posthypophysial portal system of a toad (*Bufo*). Note a large vessel between pars intermedia and pars distalis (After Rodriguez & Piezzi 1967, modified)

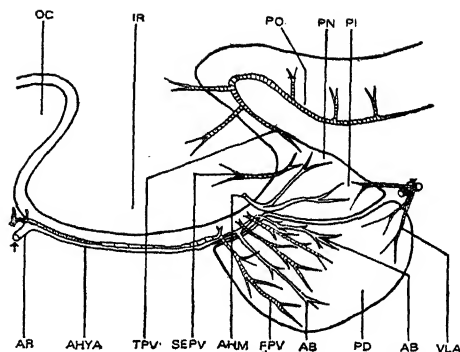


Figure 6 Encephalo-posthypophysial portal system of a toad (*Bufo*). Note the arterial supply to the pars distalis (After Hasegawa 1969, modified)

may not be wrong to assume that its blood may carry some of these secretory substances.

Primate Portal System: In the Primates including man (Daniel 1966) where there is a stalk (figure 7) (upper and lower infundibular stem) connecting the brain and the PG, a superior hypophyseal artery brings the blood into the stalk and the long portal veins formed take the blood from here into the PD. An inferior hypophyseal artery, also a branch of the internal carotid artery like the superior enters the stalk region from the lower end and short portal veins formed from these carry the blood into the lower part of the PD; the inferior hypophyseal artery also irrigates the pars nervosa (PN) and neurosecretion may be brought to the capillary bed in this region. There are veins taking away the blood from the PD into the general body circulation. Stalk section both in the monkey and human pituitaries caused a large area of necrosis and a small area along the dorsal border remains unaffected. A very large traumatic rupture above the superior hypophyseal artery level does not interfere with blood supply from superior hypophyseal artery and therefore, no infarct develops in the PD.

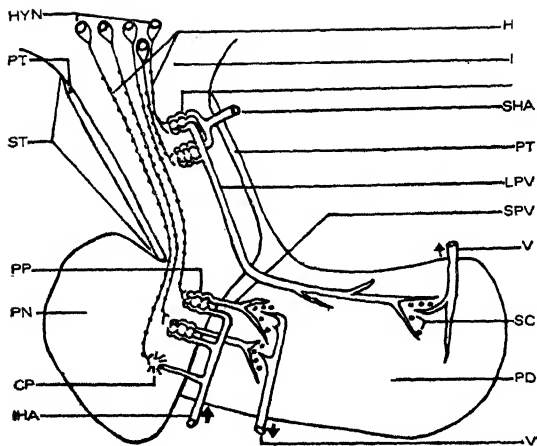


Figure 7 The long and short portal vessels to the human hypophysis, diagrammatic. The neurosecretory axons are shown to carry secretion to the primary plexus in the stalk; it may also be carried to the capillary bed in the pars nervosa. Specific neurosecretion is believed to go to specific groups of secretory cells (After Adams et al. 1964, modified)

If the stalk is severed in its middle, the long portal vessels are disconnected and a large part of the PD does not get its blood supply. Blood supply from the inferior hypophysial artery continues to take place.

Two sets of portal vessels (the long and short) then enter the PD and end up near specific cell groups which secrete specific hormones; localization of cells secreting one type of hormone appears to be characteristic of the primate pituitary. Probably specific groups of neurosecretory neurons send specific hypophysiotropins to the groups of adenohypophysial cells through the portal veins. There is no direct vascularization of the PD like that in the rabbit where on one side, a branch of the internal carotid artery vascularises the PD (it is not clear if such a direct arterial vascularization is seen in the cat, dog and monkey) in addition to the portal supply. In this rodent the PD undergoes no necrosis

on a stalk section; it is not known if it has short portal vessels (Daniel 1966). But according to Harris (1947) PG is undamaged by infarction following stalk transection but soon becomes functionless.

It has been shown (Adams et al. 1964, 66) that there is little mixing of portal blood entering the PD. As said above, if specific groups of neurosecretory cells send their secretion through the portal blood into specific areas of the PD, individual groups of cells of the supraoptic and paraventricular nuclei (SON & PVN respectively) (vide infra) may have specific secretory activity. This observation becomes very significant in view of the fact that the Milan School has described PVN synthesizing follicle stimulating hormone releasing hormone (see chapter on releasing and inhibiting hormones).

In the rat (Landsmeer 1963) also a localized area of the portal system is described to supply a specific area of PD. The different portal vessels appear to supply blood to different zones of PD.

In the rat (Daniel & Prichard 1956) there is a capillary net on the PN; from this net arise neural lobe portal vessels which penetrate through the PN and enter the PD where they break up into sinusoids; this is also described as the 'short' portal system.

Development of the Portal System

The portal capillary loops appear in the ME 5 days postnatally in the rat (Campbell 1966) and 10 days prior to birth in the rabbit. Probably neurosecretory activity also starts postnatally when monoamines appear during the first and second week postnatally. However, according to Kobayashi et al. (1968) electron dense particles are present in axon terminals in 20-day foetal rats. Even though structures appear present for neurosecretion to

pass into portal blood in the ME, its maturation takes place postnatally. The ependymal-axonal relations are still immature in the just born rats (Monroe et al. 1972).

The Neurosecretory Nuclei in Lower Vertebrates

Now the seat of all these activities may be examined. The hypothalamus shows one pair of Gomori positive nucleus [chrome-alum-haematoxylin (CAH) and AF positive] [the caudal neurosecretory system of fish is Gomori negative but all the same it is neurosecretory (Fridberg & Bern 1968)] — the preoptic nucleus (so called because it is in front of the optic chiasma) in the Amphibia and two pairs—the supraoptic (SON) and paraventricular (PVN) nuclei in the Amniota. There are also other important centres of neurons in the anterior, medial and posterior hypothalamus of Amniota but they are CAH and AF negative. In the fish generally there are two nuclei—the preoptic nucleus (PON) and the lateral tuberal nucleus (LTN); the latter is homologous with the arcuate nucleus of Amniota. In a number of fish (Charlton 1932) including *Phoxinus* (Scharer & Scharrer 1954) there is no LTN. A LTN has not been identified below Holostei (Scharrer & Scharrer 1954); it appears prominently in *Amia* and *Lepisosteus*. The PON of fish and frog is composed of two groups of neurosecretory neurons, viz., pars magnocellularis (PPM) and pars parvocellularis (PPC). The Scharrers (1954) pointed out that the fish PON is homologous with the SO and PV nuclei of higher forms, the latter two being developed due to neurobiotactic forces.

The Neurohumor

In addition to the neurons producing neurohormones and transmitting them by axons, there are others in the CNS whose

axons release chemicals such as acetylcholine, noradrenaline or norepinephrine (a precursor of adrenaline or epinephrine—also a unique secretory product of some chromaffin cells of adrenal medulla) or serotonin (5-Hydroxy-tryptamine, 5HT) which are local or diffusion hormones producing response in the effector. These chemicals are called neurohumors and they are destroyed very quickly. The term neurohumor may be included under neurohormones (Barrington 1963).

Modes of Neurosecretion

The release of neurosecretion may follow one of the following eight methods:

1. Into the ventricular cavity in between the ependyma cells, the dendrites may throw the secretion (Amphibia),
2. Into the primary plexus of the portal system in the ME or into the plexus in the PN (the latter is the second neurohaemal organ),
3. At the parenchyma cells of the PI, some fibres may enter through the PN in Anura; in *Hyla* (Smoller 1966) the fibres do not synapse with the cells. This type of 'neurosecretomotor' innervation has also been described in the crocodile (Gabe & Rancurel 1958),
4. Into the neurointermediate lobe of elasmobranchs making synaptic contacts (Knowles 1963),
5. In the eel (Knowles & Vollrath 1966), it is noticed that if the fish is placed in an illuminated background, a message is transmitted to the PON, the information is passed down the axon and this makes contact, which has all the morphological features of a synapse with a

blood vessel and also with a pituitocyte (a modified glia cell). The neurosecretion passes from the blood vessel into the adjacent melanocytestimulating hormone (MHS) cell which causes colour change. The pituitocyte appears to secrete a substance which enters the cerebrospinal fluid (CSF) and may come in contact with the dendritic neuron and thus a feed-back is established. (The figure of hypothalamo-hypophysial system in this eel is taken from Leatherland et al. (1966) with modification),

6. Chemical agents may diffuse into adjacent gland cells from a neurosecretory neuropile (Herlant-Meevis 1965),
7. There may be unidirectional flow of blood bringing neurosecretory material (NSM) from the neurohypophysis into PD [in *Ambystoma* (Green 1966) the mantle plexus penetrates into the neural lobe and then flows into PD] or into the PI (in figure 2, p. 29), the vascular link between the PN and PI is clearly shown (Green 1947); Heller and Ginsburg (1966) also feel that there is a vascular link between PN and PI. Greep (1963) also reproduces figure 2 referred to above in his paper on adenohypophysis vasculature (figure 7),
8. It has also been shown in *Lepidostiren* (Zambrano & Iturriza 1973) that the peptidergic neurosecretory axons coming from PON and adrenergic neurosecretory axons coming from the small neurons of the ME may end up on the connective tissue between the PN and PI or between the posterior part of the ME and PD.

Neurosecretion in Fishes

The Neurohypophysis and Portal Circulation.

The relationship between the neurohypophysis and the portal circulation has been recently reviewed by Perks (1969).

In *Petromyzon* (Oztan & Gorbman 1960) (figure 8), the floor of the infundibulum is thin and is connected with the underlying PG by a set of blood vessels; the PG has been identified as rostral PD, proximal PD and PI. It is not clear if the rostral PD answers to a PT (Bern & Nandi 1964). The PON (Oztan & Gorbman 1960) sends bundles of fibres posteriorly; one bundle goes to the hindbrain. A set of fibres passes laterally and enters the neurohypophysis. The third set of fibres passes close to the floor of the infundibulum and enters the neurohypophysis. There is also a posterior hypothalamic neurosecretory nucleus from which fibres may go to the neurohypophysis (?) and to the midbrain. It has been opined that blood vessels connecting the floor of the infundibulum and rostral

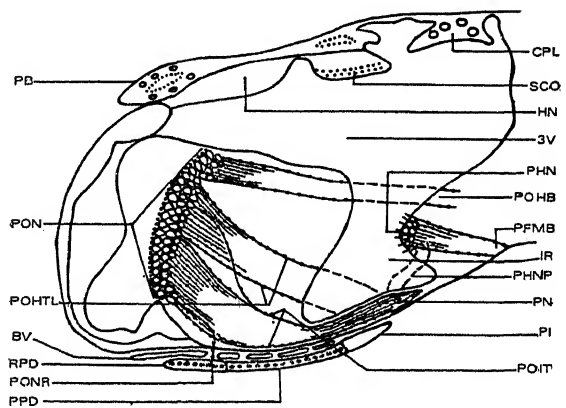


Figure 8 The hypothalamic PON fibre tracts and parts of the pituitary in *Petromyzon*, diagrammatic (After Oztan and Gorbman 1960, modified). The labelling of parts is according to Gorbman and Bern (1962)

and proximal PD answer to a simple portal system and the same floor to the ME; a PN is not developed.

In *Polistotrema*, also a cyclostome, Gorbman et al. (1963) have described a portal system between the anterior diencephalic floor and the *neurohypophysis*. No vessels or blood from the neurohypophysis appear to enter the adenohypophysis. A hypothalamo-adenohypophysial portal system is absent.

In *Myxine* (Olsson 1959) (figure 9), there are two preoptic nuclei on either side; the more dorsal of them; the magnocellular sends fibres towards the infundibulum—the preoptico-infundibular tract; a few of these fibres end in a specialised anterior floor of the infundibulum—the 'median eminence'. While Olsson (1959) did not describe a portal circulation or a vascular connexion between the infundibulum and the adenohypophysial islets situated below, Gorbman (1965) described a hypothalamo-neurohypophysial portal circulation in *Myxine*.

In the Elasmobranchii (*Scylliorhinus*) (Knowles 1965) neurosecretory fibres arise from the PON and terminate in the neurointermediate lobe or PI. These are the type A fibres. These fibres contain elec-

tron dense vesicles (1800 Å) which are CAH positive. These seem to act locally and carry oxytocic properties. Mellinger (1963) also described in *Scylliorhinus* a set of fibres comparable to type B of Knowles (1965) arising from LTN with electron dense vesicles less than 1000 Å, CAH and alcian blue negative; a few of these fibres terminate in the ME and others go to the neurointermediate lobe. These probably carry catechol hormones (Knowles 1965).

The hypothalamus appears to have an inhibitory control over the MSH of the neurointermediate lobe. The B-type of fibres regulate the release of MSH. Both types of fibres make secreto-motor junctions with cells of neurointermediate lobe (Knowles 1965).

According to Mellinger (1960, 1963) there are well developed hypothalamo-hypophysial portal vessels coming from the two media eminentia in *Scylliorhinus* (see his figure 112, Thesis 1963). According to Meurling (1960) the single ME (figure 10) not only sends a few portal vessels into pro- and meso-adenohypophysis but mostly into the neurointermediate lobe (*Scyllium*, *Raja*), a unique feature noticed in a few other vertebrates like *Myxine*, *Polistotrema* ("probably the most

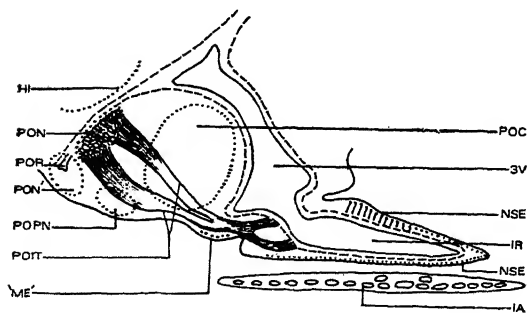


Figure 9 The hypothalamic PON fibre tracts and adenohypophysis in *Myxine*, diagrammatic. Note the division of the PON into groups and the so-called 'median eminence' (After Olsson 1959, modified)

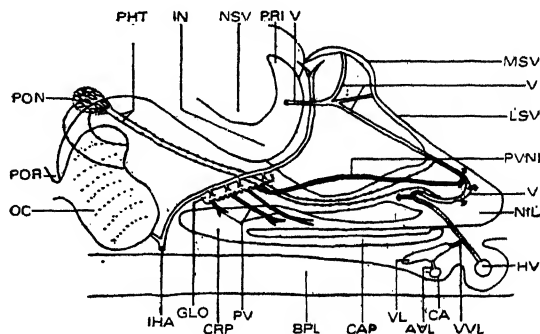


Figure 10 The hypothalamo-neurohypophysial pathway and vascularization in a dogfish. Note a portal vessel going to the neurointermediate lobe, diagrammatic (After Mellinger 1960, modified)

primitive of living vertebrates"), *Hydrolagus* and *Chimera* (Holocephale).

We (Lazarus and Ramaswami, unpublished observations) have examined the hypothalamo-hypophysial system in the Indian shark *Scoliodon sorrokowha*. In this, the hypothalamus (figure 11) shows an anterior thicker portion and a thin posterior portion. In the region of the optic chiasma, the small neurons of the PON are seen; these are CAH positive. Posterior to the chiasma, similar small neurons of the nucleus lateralis tuberis (NLT) are seen; these are also CAH positive; the fibres from these nuclei appear to pass into the posterior thinner portion of the hypothalamus; the neurosecretory fibres then enter the PI from this posterior part which can be clearly made out in the preparations. In addition, blood vessels from the external wall of the anterior portion of the hypothalamus enter into the dorsal PD (rostral and proximal parts of PD); these probably bring in neurosecretion into the PD of the adenohypophysis. Blood vessels from the thin posterior part of the hypothalamus also enter the PI; this is probably portal irrigation of the PI. It is very unusual that portal blood should irrigate not only the PD but also the PI, a situation also noticed in the hagfish (Gorbman 1965) and a few other fish previously stated.

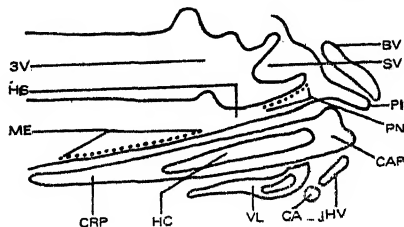


Figure 11 Longitudinal sectional view of the hypophysial region of a juvenile Indian shark *Scoliodon* to show its anatomy. The ventral lobe has its own cavity unconnected with that in the pars distalis, diagrammatic (After Lazarus and Ramaswami, unpublished)

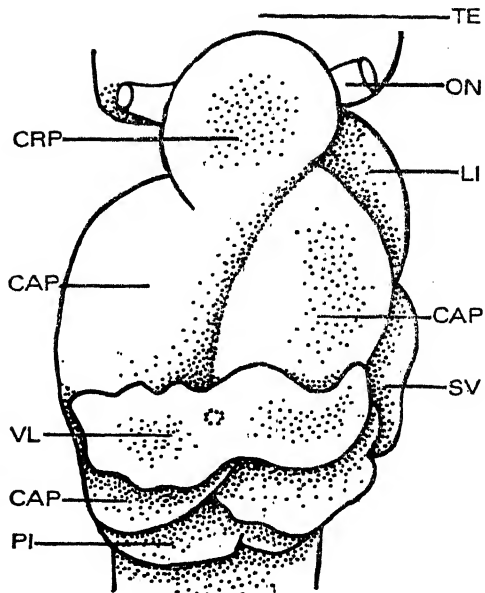


Figure 12 Anatomy of the pituitary gland of *Scoliodon*, ventral view, (slightly tilted, after Lazarus and Ramaswami, unpublished)

Jørgensen (1968) feels that the blood vessels entering the PI in this group of fish may not be carrying any neurosecretion into the lobe.

The PG of *Scoliodon* (figure 12) referred to above also shows a ribbon-shaped ventral lobe (of the PD) connected with the posterior part of the dorsal PD by a cord of cells; the vascularization of this part appears to be directly from the carotid artery; in *Raia*, some portal blood seems to enter the ventral lobe (Chevins 1968, quoted by Ball & Baker 1969).

In *Hydrolagus* (Holocephale) according to Sathyanesan (1965) there is a functional ME and a portal system; no portal blood goes to the neuro-intermediate lobe as in the elasmobranchii. The NLT contains AF positive secretion. Jasinski and Gorbman (1966), however, describe also a hypothalamo-neurohypophysial portal system in *Hydrolagus coliei*.

In the Polypteriformid *Calamoichthys* and *Polypterus* (Lagios 1968) there is a ME and a portal circulation as also in *Acipenser* (Polenov 1966) (figure 13): in both the above orders Polypterini and Chondrostei to which the above named fish belong respectively, the hypothalamus lacks a LTN.

In the Dipnoi, the PN may send finger-like processes into PI (*Neoceratodus*, *Protopterus*) (Kerr & van Oordt 1966) but not in *Lepidosiren* (Zambrano & Iturriza 1973) (figures 14, 15). In *Protopterus* (Kerr & van Oordt 1966) each hypothalamo-hypophysial tract divides into an upper

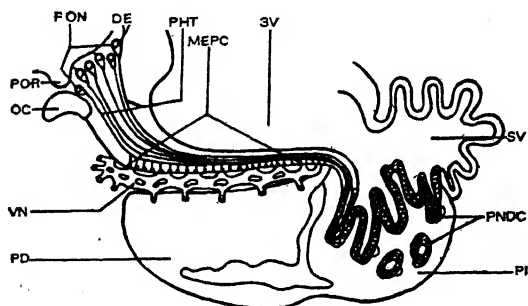
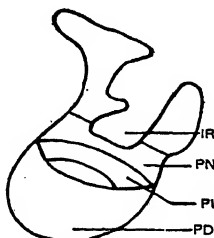


Figure 13 The hypothalamus and pituitary gland of *Acipenser*, diagrammatic. Note the presence of a portal circulation (After Polenov 1966, modified)



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Figure 14 The pituitary gland of *Protopterus*, diagrammatic. Note the pars nervosa sending processes into the pars intermedia (After Romer, see Gorbman & Bern 1962)



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Figure 15 The pituitary gland of *Lepidosiren*, diagrammatic (After Romer, see Gorbman & Bern 1962)

and a lower part; the latter supplies the ME capillary plexus and a few beaded axons reach the front of the nervous lobe. The larger upper part goes to the PN. In *Lepidosiren* (Zambrano & Iturriza 1973) there are two sets of neurosecretory fibres; one set, the peptidergic, arises from the PON. The main bundle of this hypothalamo-hypophysial tract passes through the lateral wall of the infundibular cavity and terminates in the PN. Fibres of this peptidergic tract also go to portal vessels, to cells in the PI and to the connective tissue in between the PD and the posterior part of the ME. The second set of fibres arises from small neurons of the ME and proceeds to the PD and PI cells and also to the connective tissue in between the PN and PI.

The peculiarities of the dipnoan hypothalamo-hypophysial system may be summarised. The PON is short when compared with the teleostei; the ME is innervated by a special ventral neurosecretory tract; the PN is invaded by the infundibular recess and there is a portal system draining into the PD (Perks 1969).

Incidentally, it may be noted that the PN has appeared for the first time in Dipnoi; however, it has already been said that some elasmobranchs at least show a structure similar to the PN. Heller (1964) however, noted that 'an independently vascularised "neural lobe" develops only in amphibians'.

The teleostei are very different from the previous groups studied. According to one set of observers (see Perks 1969), a ME and a portal circulation is absent. However, Kobayashi and Wada (1973) note (p. 289) that "In fishes, the neurosecretory cells send their axons mostly to the posterior part of the neurohypophysis, which is equivalent to the PN of higher vertebrates but a few axons terminate in the anterior part, which corresponds to the median eminence".

Generally in teleosts, as seen in *Anguilla* (Knowles 1963, Knowles & Vollrath 1966) and *Leuciscus* (Samuelson et al. 1968) the fibres of the PON terminate in the PI (meta-adenohypophysis or neurointermediate lobe) and those of the NLT in the rostral (which probably corresponds to the PT) (Bern & Nandi 1964) and the proximal PD (which corresponds to the entire PD of tetrapoda) (Bern & Nandi 1964) (pro- and meso-adenohypophysis) (see figure 16). In the eel, Knowles and Vollrath (1966) do refer to a distal ME the fibres penetrating into the adenohypophysis) and a distal portal circulation (the perivascular and intervacular spaces) (p. 339). In *Phoxinus* where an NLT is absent, the preoptic fibres innervate the meso- and meta-adenohypophysis.

Both the Gomori-positive preoptic neurosecretory fibres, and the Gomori-negative fibres coming from the NLT end on or near the epithelial cells of the PI or meta-adenohypophysis (Legait & Legait 1958). The proximal PD may also be innervated by the neurosecretory fibres. Neurosecretory

fibres also reach capillaries of the pro- and meso-adenohypophysis (figure 9) (Follenius & Porte 1962); the NSM reaches the superficial venous system. This neurovascular system plays a part similar to the portal system of higher vertebrates which is lacking in most teleosts (Follenius & Porte 1962).

It is now established that the neurosecretion from the NLT is different from that of PON. In *Salmo* (Follenius 1962) the NLT neurons actually penetrate into the pre-optico-hypophysial tract and their neuroaxons reach the PG. The elementary vesicles found in the axons of the NLT are smaller and stain differently from those of PON. The nucleus shows seasonal activity. The hormone coming from PON is concerned with salt and water balance while that from NLT controls reproduction (Jørgensen & Larsen 1967).

In *Phoxinus*, Barrington (1960) described a vascular pattern in the floor of the infundibular recess resembling a ME; however, the secretory cells here transport the products into the ventricle thereby differing from the conventional ME of tetrapods. Having studied the NLT during resting and spawning season in *Clarias*, Dixit (1967) reported a cyclical activity in its neurosecretory mechanism. It has been brought out that the type B fibres coming from the NLT of *Gillichthys* mediate in pituitary gonadotropin regulation (Zambrano 1971); the same author has shown previously that in this fish the NLT shows histochemically catecholamines (Zambrano 1970).

It has been brought out (Stahl & Leray 1962) that the NLT neurosecretory material has no axonic pathway; the material flows into the CSF and the ependyma absorbs this and passes it on to the capillaries from where it goes to the meso-adenohypophysis. According to the same authors, the NSM is CAH, 'AF' and 'AT' ne-

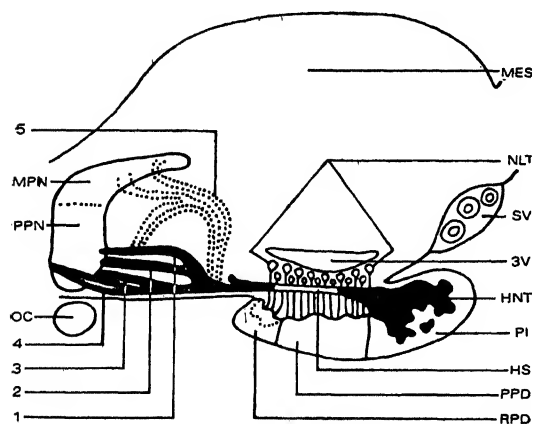


Figure 16 The hypothalamus and pituitary gland of *Anguilla*, diagrammatic. The hypothalamo-neurointermediate tract is formed by 5 different strands. (After Leatherland et al. 1966, modified)

gative but shows annual cyclic activity in the adult. Ortman (1960) has also brought out that in fish, axonic transport of NSM has not been seen in the NLT; the NSM in fish, Amphibia and also mammals may find its way into the CSF and is described as hydrencephalocrinie; the cell nucleus may also contribute towards the formation of neurosecretion.

Sathyanesan (1969) studied the neurosecretory tract of *Clarias* by the bulk-staining technique. If the hypothalamo-hypophysial stalk is severed, a neurohypophysis-like organ develops at the severed end which could also be followed by the bulk-staining technique. While he refers to two tracts from the supraoptic nuclei which unite to form the infundibular stalk ending up in the PI, he does not refer to the fibres coming from the LTN as these are not stained by this method. Sathyanesan (1972) also described the existence of a portal system and two media eminentia. The anterior ME is comparable to the tetrapod one; the blood vessels form a primary plexus in this region. Some neurosecretory axons appear to end at this region and the axon terminals and blood vessels are in close apposition. The primary plexus forms portal vessels and these irrigate the PD (rostral and proximal) and the PI. The portal vessels also form an interface capillary plexus in the rostral neurohypophysis and this interface vasculature before it irrigates the rostral PD brings some neurosecretion from the associated Herring bodies. This region also therefore acts like a ME and is probably the typical teleostean ME according to the author.

A similar state of affairs is also described in *Heteropneustes* by Sathyanesan and Haider (1971). However, Sundararaj and Viswanathan (1971) also describe the hypothalamo-hypophysial system of the same fish and state that the axonic fibres

carrying the neurosecretion from the nuclei may end near the cyanophils of the pituitary sinusoids thus carrying the chemicals to the cells. Further, according to the same authors, the incipient vascular connexion between the ventral hypothalamus and the PG described by a number of workers in fish does not constitute a portal system and therefore, there is no portal system in the catfish. They have also brought out that the amount of secretion in the PON undergoes cyclical changes with reference to reproduction. The fibres coming from the NLT merge with those of the PON. With regard to the vascularization of the hypothalamic nuclei in this fish, the PON is supplied by a branch of the internal carotid artery and the NLT receives the ventral hypothalamic artery which is a branch of the hypothalamo-hypophysial artery.

Hyashida (1971) has brought out that if the growth hormone of a fish cross-reacts with that of a mammal, then it has a portal circulation; in *Heteropneustes*, it does not cross-react.

The eel hypothalamo-neurohypophysial system is less active in sea-water; it gets stimulated when the fish are transferred to distilled water (Hanke et al. 1969).

A study of the hypothalamo-hypophysial system has brought out that in the teleost fish, a portal system is poorly developed as the neurosecretion is directly carried to the adenohypophysial cells. The neurohypophysis therefore controls the activities of the adenohypophysis by the above two courses. Perks (1969, p. 170) feels 'that the median eminence became incorporated into the pituitary as the rostral region of the PN'.

The Caudal Neurosecretory System and Urophysis

Speidel (1919) described large secretory cells (the Dahlgren cells—named after its

discoverer) in the caudal part of the spinal cord of elasmobranchs. He considered them to be gland cells of internal secretion.

Enami (1955) described the caudal neurosecretory system in *Anguilla*. He pointed out that the neurons gave rise to axonic fibres carrying secretion into a neurohaemal organ. This organ—the urophysis is comparable to the PN of the neurohypophysis of the hypothalamic system. The elasmobranchs, teleosts and Chondrostei show the caudal neurosecretory system; however, the elasmobranchs lack a urophysis. The urophysis may be attached ventrally to the spinal cord or dorsally (*Stomtas*), may not be externally visible (*Anguilla*, *Esox*), may be completely absent (*Nerophis*, *Siphostoma*) or may be connected by a stalk (*Lophius*).

Lederis and Bern (see Bern 1971) suggest that there may be neurophysin-like putative carrier protein in the caudal neurosecretory system and have provisionally christened it 'urophysin'. Chan (1971) quotes that urophysectomy in *Tilapia* caused hypertrophy of PON. He also quoted the regeneration of a new urophysial system taking place about 10 days after urophysectomy. According to Chan, the active principles resemble the neurohypophysial peptides.

The ependyma seems to be the origin of Dahlgren cells; the elementary granules range from 800–250 Å; the axons may be myelinated or not and the neurosecretion is probably osmoregulatory (Fridberg & Bern 1968). The Dahlgren cells receive aminergic innervation (5 HT of catecholamines?) (Wilén & Fridberg 1969).

Neurosecretion in Amphibia

Very different from the previous group, recent researches have brought out a fund of interesting facts, particularly on the experimental side in Amphibia. Lesions in

the 'preoptic area' of *Triturus cristatus* brought about the loss of spermatogenesis indicating gonadotropic inhibition (Mazzi 1952). The same treatment of toads caused cessation of moulting (Scharrer 1934). The extirpation of PON in *Rana temporaria* led to the disappearance of the ME, neurosecretory fibres and the neural lobe (Dierickz 1963). Srebro (1970b) conducting the same type of experiment in *R. temporaria* and *esculenta* as done by Dierickz (1963) found the atrophy of the neural lobe, reduction of ME and the hypertrophy of the PI under the same circumstances.

According to early descriptions (Bargmann 1949), the classical 'neurosecretory system' consisted of the neurosecretory tracts starting from the PON and proceeding dorsally to the optic chiasma and then entering the first stalk (also described as the hilar region of the infundibulum) and then reaching the PN by proceeding through the ME and a second stalk. More recent staining techniques have disclosed that a few nerve fibres of the tract end near the portal vessels of the ME. The tract in the ME runs in the layer in contact with the ependyma (Rodríguez 1969). A few fibres from the second stalk may also reach the PI as the latter is in close contact with the stalk (seen clearly in *R. catesbeiana*, *Bufo*) and establish direct secreto-motor contact with the gland cells (Knowles & Bern 1966) as vascularization of this part is poor.

The Preoptico-hypophysial Tract

The formation of this tract differs in the Urodela and Apoda on the one hand and Anura on the other. In the Anura (*Rana*, *Bufo*, *Hyla*) (Gabe 1966) the preoptic neuraxons form two fasciculi, one of which runs "ventrally caudally and laterally, bends round the optic chiasma along its

dorsal border and goes directly to the floor of the third ventricle. The second fasciculus first runs rostrally, ventrally and laterally, reaches the base of the brain in a transverse plane rostral to the optic chiasma and then, making a fairly acute bend, runs caudally. The two fasciculi unite again in a transverse plane which more or less corresponds to the caudal border of the optic tract and enter the infundibular lobe". In the Urodela according to Gabe (1966) such a formation into two does not occur. In the frogs and toads examined by me the formation of two fasciculi is also noticed; the fibres running in front of the PON towards the preoptic recess is easily made out and contributes towards the formation of one fasciculus. I have not noticed such a division into two in the urodel (*Tylotriton*) and the apods (*Ichthyophis*, *Uraeotyphlus*, *Gregeneophis*) examined by me.

In the frog, according to Dierickz (1967), the PON (figure 17) is situated in the wall of the preoptic recess; the neurons are of the magnocellular type. From the ventral part of the nucleus, axonic fibres arise and pass dorsally to the optic chiasma and then descend into the infundibular floor and proceed to the PN where the axons terminate near the capillaries. These fibres are AF and PAS positive and form the preoptico-neurohypophysial tract (pep-

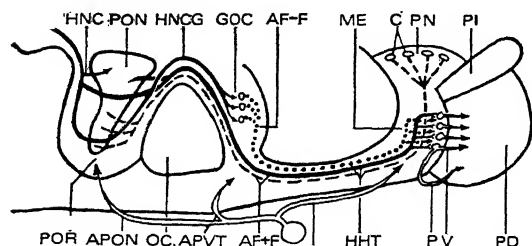


Figure 17 The hypothalamo-hypophysial neurosecretory pathways and vascularization in a frog, diagrammatic (After Dierickz 1967, modified)

tidergic); this is involved in water balance. Knowles (see discussion on p. 822 in Bern 1971) defines peptidergic nerve as having vesicles of more than 100 nm diameter as Gomori and AF positive. The dorsal region of the PON gives rise to axonic fibres which also follow the preoptico-neurohypophysial tract; when they reach the ME, they end near the capillaries there. These fibres are AF positive but PAS negative and are related to sperm release. In addition, according to Dierickz, there are certain fibres coming to the pars ventralis tubercis as higher neural control of the gonadotropic centre. Certain neurons of this centre send AF negative axons to the ME where they end at the capillaries. This tract of fibres and the above described preoptico-neurohypophysial tract together form the hypothalamo-hypophysial tract.

The Aminergic Tract

In addition to the peptidergic tract described above, in the frog the disposition of aminergic tracts in the hypothalamus has been studied (Prasada Rao 1975). On both sides of the preoptic recess (figure 18) bordering the lamina terminalis and the nucleus preopticus, the preoptic recess organ is noticed. The latter organ is a concentration of neurons and they may be

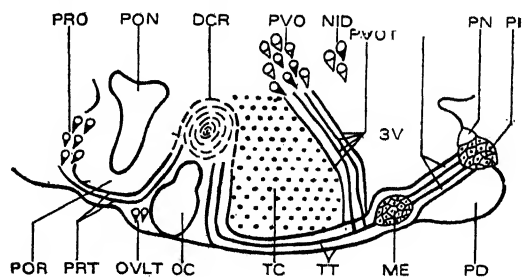


Figure 18 The aminergic pathways in frog, diagrammatic. Note the double tract from median eminence to the pars intermedia (After Prasada Rao 1975, modified)

located intraependymally or hypendymally. Falck-Hillarp fluorometric study disclosed the presence of dopamine (DA) in the neurons of the preoptic recess organ.

Forming part of the PON, catecholamine producing neurons were described by Braak (1970). These are separate from the magnocellular part of the PON and may be called nucleus preopticus periventricularis. The fibres coming from this nucleus contain monoamine oxidase. A third centre is the organum vasculosum laminae terminalis located in the ventromedial corner of the preoptic recess. The fibres from this probably join the tract coming from the preoptic recess organ.

At the border between the thalamus and the hypothalamus of the caudal hypothalamus, there is a group of neurons. The apical processes of these neurons penetrate the ependyma and form an intraventricular plexus (Braak & von Hehn 1969). The neurons proceed in a ventrocaudal direction building up the paraventricular organ (see figure 30) and the nucleus infundibularis. The term paraventricular organ was introduced by Vigh et al. (1967).

The nucleus infundibularis dorsalis has neurons similar to those of the paraventricular organ and send protrusions into the CSF. This group may be homologous to the infundibular nucleus or the arcuate nucleus of mammals. The paraventricular organ and the nucleus infundibularis dorsalis send fibres to the PI and are involved in the control of MSH secretion.

The paraventricular organ and the nucleus infundibularis dorsalis indicated fluorometrically the presence of DA and 5-hydroxytryptamine and/or 5-hydroxytryptophane in them (Rodriguez 1969).

The PI disclosed both dopaminergic and noradrenergic innervation.

Kobayashi et al. (1965) describe in the bull frog axon fibres from certain non-

neurosecretory neurons (the exact location of them is not known) pass into the hilar region of the infundibulum and synapse with secretory axons; a few of these non-neurosecretory fibres may proceed to the capillaries in the PN (figure 19) and a few may also end up near the primary plexus of the ME. Kobayashi et al. (1965) believe that the neurosecretory storage organ is controlled by these synapses; this method appears to be basic in vertebrates. No neurosecretory or non-neurosecretory fibres are shown (figure 19) proceeding to the PI.

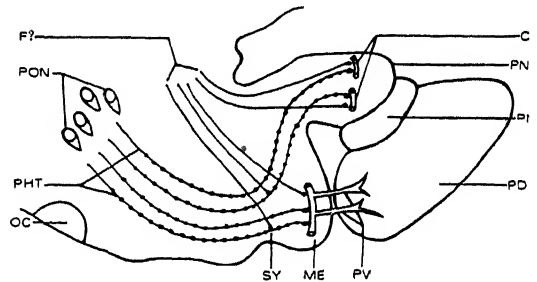


Figure 19 The neurosecretory pathways and other fibre tracts probably aminergic, in the toad, diagrammatic (After Kobayashi et al. 1965, modified)

In the light of recent researches, the fibres referred to above are probably aminergic ones coming from the paraventricular organ as shown in figure 30. Kobayashi and Wada (1973) show both in the fish and Anura certain Gomori negative fibres coming from the neurons (whose exact location is still doubtful) and ending near the blood vessels of the ME; these fibres are supposed to carry the releasing or inhibitory factors. That part of the ME in close contact with the PD shows glia cells, portal capillaries and terminations of some neurosecretory axons. In the Anura and the majority of Urodela there

are therefore, two neurohaemal organs,—the PN and the ME.

Development of the Preoptic Nucleus

The observations of Weber as early as 1965 on larval *Salamandra* PON opened up a new chapter with regard to the functional importance of this nucleus. He showed that there was a close correlation between the development of PIC (pseudo-isocyanine) positive cells in the dorsal PON region and the pituitary TSH basophils and thyroid cells in the larva. This has been subsequently shown to be true of anuran tadpoles also. Based on their own work and on previous studies van Oordt et al. (1972) suggest that PIC positive dorsal region of the PON is the seat of TRF synthesis. According to Chelverukin (1971) it has been noticed in *R. temporaria* that during ontogenesis, the PON arises in two stages, the neurons of the dorsal region of the nucleus differentiate earlier than those of the ventral. Prior to their differentiation, increased mitotic activity is noticed in the ependyma bordering the 3rd ventricle and its preoptic recess. During early stages, no neurosecretory material is noticed in the preoptic neurons or their tracts or in the future ME but only in the PN. The NSM appears in the PON during and after metamorphosis particularly in the dorsal preoptic region when the other regions also show the same.

Interesting observations have resulted from a study of the ontogenesis of *Xenopus laevis* (van Oordt et al. 1972). At Stage 58, both peptidergic (stained by AF and PIC techniques) and aminergic by Falck-Hillarp (FH) techniques have been done.

The Peptidergic System

This consists of the PON divisible into

dorsal and ventral portions located, as previously stated, in the preoptic recess and can only be demonstrated by using PIC and the tracts emanating from them. Further in the larvae of *Alytes*, Disclos (1967) brought out that the PIC positive tracts went up into the telencephalon and into epiphyseal and habenular reaching into the mesencephalon.

Peculiarly in the hypothalamic hypophysiotropic area of *Xenopus*, three pairs of nuclei are seen: (1) the paraventricular organ having two types of cells both with their processes in contact with CSF; these processes seem to be both secretory and sensory. The paraventricular organ is aminergic monoamine containing nucleus and is FH positive. The cell bodies contain dense core vesicles 800 and 1200 Å in diameter; (2) the nucleus infundibularis dorsalis (NID) is situated in the dorsal wall of the recessus mammillaris. Two types of cells are found making contacts with the CSF as above. The dense core vesicles are of 900 and 1300 Å in diameter. This is also aminergic monoamine containing nucleus and FH positive; (3) the nucleus infundibularis ventralis (NIV). This is also situated subepithelially as above of the infundibular recess and has two types of cells. These cells are not FH fluorescent and are not stained with AF or PIC. Ultrastructurally the cells answer to a peptidergic nucleus with granules of 1400 and 1800 Å in diameter. Fibres emanating from these nuclei are not clear. As previously pointed out, the paraventricular organ fibres seem to end in PI and probably cause release of MSH. The NID fibres may go to PI and also end near the portal vessels of ME. Probably NIV fibres cause gonadotropic release.

The Portal System

India ink injected frogs and toads dis-

close the disposition of the vessels on the infundibulum, ME and the PG. A posterior branch from the infundibular artery on either side proceeds towards the ME and forms a rich plexus there; the capillaries enter deep into the issue. The disposition of branches on either side is not always symmetrical. The PI also receives the same artery irrigating it as the one to the ME and forms a rich plexus. From the ME, portal veins enter the PD and form a secondary plexus. In the urodel *Tylotriton* studied by me, I am unable to describe the portal system as I do not have injected specimens. In *Ichthyophis* and *Gegeneophis*, a ME is prominently present and blood vessels are noticed in it (see Pl. 2, figure 26). Until an India ink injection study is made and proved otherwise, it may be inferred that the presence of a portal circulation is likely in the apods.

Certain Peculiarities of Neurosecretion

Recently it has been reported in metamorphosed toads (*Bufo*), that AF positive granules enter directly from the ME into the PD (Dixit 1973).

In *R. pipiens* nerve fibres may carry neurosecretion from the PN into the PI (Dawson 1953). Wingstrand (1966) clearly shows in figure 8 (p. 15) nerve fibres entering from PN into the PI. Such a phenomenon has also been noticed in fishes and mammals (Jørgensen & Larsen 1963). It has also been brought out that the PI is controlled by direct innervation (Jørgensen & Larsen 1963) and Etkin (1962) thinks that these nerves are of an inhibitory nature.

In the more aquatic Amphibia (Urodela), a ME is not developed as e.g., *Necturus* where the vascular connexion is between the infundibulum and the PD (Green & Maxwell 1959); in others like *Ambystoma*

there is a ME but there does not seem to be a portal circulation. In the only Indian urodel *Tylotriton verrucosus* (figure 20); (Pl. 1, figures 21, 22) studied by me, the infundibular floor is not developed into a thickish ME as seen in *Rana* but remains thin (thick arrow) as in *Triturus* (see Green & Maxwell 1959). In *Tylotriton* fuchsinophilic neurosecretion is noticed in the region of the ME adjacent to the PD (figure 22, PD) and in this region rich capillary plexus (thin arrow) is noticed suggesting that neurosecretion may pass through these capillaries into the PD; however, the capillaries did not disclose any AF positive secretory granules though granules are noticed in ME adjacent to the capillaries (external layer) and these could be traced into the PN. The latter is intensely AF positive and is highly vascular. The pars intermedia (figure 20), (Pl. 1, figure 21, thin arrow) is clearly demarcated and exhibits a few

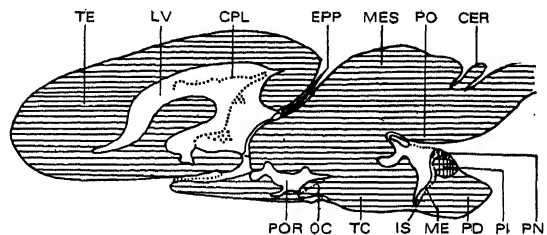


Figure 20 Longitudinal sectional view of *Tylotriton verrucosus* brain showing the hypothalamus and the pituitary gland. Note the median eminence is in close contact with the pars distalis and that there is a well developed pars intermedia (unpublished), CAH ($\times 200$)

AF positive basophils but is not vascular. Under light microscope studies, fibres from the PN were not seen entering the PI.

Median Eminence in Anura

Kobayashi et al. (1970) described that in the Anura, the ME shows a well developed



Figure 21 The median eminence and pituitary gland of *Tylotriton*, longitudinal section, CAH-phloxine ($\times 200$). The median eminence (thick arrow) is in close contact with the pars distalis (arrow-head); the pars nervosa is intensely stained and between it and the pars distalis is the pars intermedia (thin arrow)

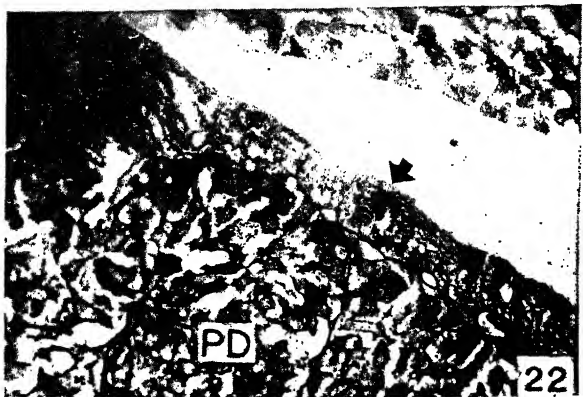


Figure 22 Magnified view of the pars distalis and the median eminence (thick arrow) as in figure 21. Between the median eminence and the pars distalis can be seen capillaries (thin arrow) and these probably carry neurosecretion into the pars distalis. Portal veins as such could not be made out



Figure 23 Enlarged view of the pituitary gland of *Ichthyophis glutinosus*, CAH-phloxine longitudinal section, approximately ($\times 1025$). The pars nervosa (thick arrow) is lodged in a depression of the pars distalis (arrow-head) and the hypothalamo-neurohypophysial tract (thin arrow) passes in this animal ventrally to the pars distalis and joins the pars nervosa from its posterior end. This is probably a variation



Figure 24 The infundibular stalk, mesencephalic flexure and the pituitary gland of *Gegeophis beddomiei*, CAH-phloxine ($\times 200$), longitudinal section. Note the pars nervosa projecting like a beak (thin arrow) from the posterior end of the pituitary gland. The hypothalamo-neurohypophysial stalk passes dorsally into the pars nervosa through the pars distalis (arrow-head)

secretory ependymal layer from which processes proceed towards the external layer. Hypendymal cells are very sparse and then there is a poor fibre layer. The primary plexus is situated in between the ME and the PD and sends capillaries into the parenchyma of the ME. The reticular and palisade layers do not exhibit typical avian or mammalian pattern. The Gomori positive and negative fibres and glial processes are included in the fibre layer of the ME.

In the urodelan *Triturus*, *Ambystoma* and *Necturus* where previous workers denied the presence of a ME or a portal system are now described as possessing a ME but not developed into a thickish structure as previous workers would have it, and also a portal system draining into the PD.

Another function is ascribed to the urodelan hypothalamus by Mazzi (1970). It may be described as a thermosensitive centre where external stimuli impinge upon the internal ones, get integrated and are passed on to the adeno-hypophysis by the hypothalamo-hypophysial system.

In the apodan *Hypogeophis rostratus*, Laubmann (1926) described a long infundibular stem enclosing an extension of the infundibular canal reaching into the PN; the PI surrounds the PN like a ring. Wingstrand (1966) described in the same species that the PI occupied an area between the PN and PD. In *Gegeneophis carnosus*, Pillay (1957) described that the infundibular cavity was limited to it and there is no reference to an infundibular canal extending into the PN. The stalk connecting the infundibulum and PD is flat and the PN is rhomboidal in shape situated at the extreme tip of the hypophysis. Further, according to him "There is a group of elongated cells at the anterior narrow region of the hypophysis which resembles the intermediate cells". This region he

has labelled PI in his figure 5; this region correctly is the ME. He also implied the presence of a portal system in *Gegeneophis* when he said that the blue bodies of basophil cells of PD may be brought by portal circulation from the infundibulum. Thus the neurosecretion synthesized in the PON, according to him, takes three courses: (1) entering portal circulation and reaching its destination, (2) entering CSF after becoming extracellular and (3) stored in the PN. Wingstrand (1966) however, referred to the presence of a ME and a portal system in *Gegeneophis* basing his observations on the doctoral thesis of Pillay (University of Trivandrum, 1961). Pillay (1957) described the PON as occurring on either side of the 3rd ventricle (figure 3). A peculiar feature noted by him is the union of axons of a few neurosecretory cells to form a common axon (figure 4b). The neurosecretion in *Gegeneophis* is of two kinds: (1) the juxtannuclear CAH positive granules which are carried to the PN; (2) the CAH positive colloid type (mulberry-like) is probably extruded into the CSF after becoming extracellular.

Gabe (1972) working on *Ichthyophis glutinosus* noted that the PN is flat; the PI surrounds laterally the PN. Further, the PD sends two projections anteriorly corresponding to the PT. As he did not do any injections, he was not sure of a portal system; all the same he referred to the presence of a ME and remarked on the wrong identification of ME as PI in *Gegeneophis* by Pillay (1957). With regard to the PON, Gabe described that it was noticed on either side ventrally of the 3rd ventricle in the region where dorsally the subcommissural organ was present. These secretory cells extend considerably posteriorly. Dendrites from the neurosecretory neurons proceed, towards the 3rd ventricle and as happens in Anura, may throw the secretion into the

CSF. In *Ichthyophis*, the PON is not differentiated into magno- and parvo-cellular components. Further, the neuraxons coming from the PON to form the hypothalamo-hypophysial tract are not gathered into two fasciculi as happens in *Anura* before they reach the PN but resemble the *Urodela*.

I have examined the hypothalamus and the hypophysis in the south Indian apodan genera, viz., *Ichthyophis glutinosus*, *Uraeotyphlus narayani* and *Gegeneophis beddoeii* and *ramaswamii*. In all these there is a stalk extending from the infundibular tip to the PN which latter is lodged posteriorly under the pons, in a dorsal depression in the PD in *Ichthyophis* (Pl. 1, figure 23, thick arrow and figure 25) and

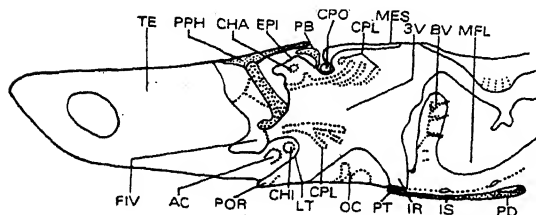


Figure 25 Longitudinal sectional view of a juvenile *Ichthyophis glutinosus* showing the hypothalamus and the pituitary gland. Note the anterior extension of the pars distalis to form the paired pars tuberalis

Uraeotyphlus while in *Gegeneophis* (Pl. 1, figure 24, thin arrow) it forms the posterior tip of it. The stalk has also the hypothalamo-hypophysial tract, mostly dorsally (figure 24, arrow-head) coming from the PON and ending up at the capillaries of the PN. Both in *Ichthyophis* and *Uraeotyphlus*, there is no extension of the infundibular cavity into the PN; in the picture (Pl. 1, figure 23) of *Ichthyophis* hypophysis, the hypothalamo-hypophysial tract runs ventrally (thin arrow) to the PD (arrow-head) and joins the PN at its posterior end; this probably is a variation

In *Gegeneophis*, the infundibular cavity extends into the PN. In *Ichthyophis*, anterior extensions of the PD to form paired PT (figure 25) are noticed, a point also referred to by Gabe (1972). Burckhardt (1891) does not show a ME or a PI in *Ichthyophis*; a PN is shown in his figure of a median longitudinal sectional view of the brain (Pl. XXI). Kuhlbeck et al. (1966) give a figure of an apod *Schistomermus* brain in which the infundibular stalk is short and there does not appear to be an extension of the 3rd ventricle into the PN. No other parts of the PG are described therein. Further, in a transverse sectional view of the brain of the same species, the authors portray the parts of the thalamus, the dorsal parapophysis and the projection of the wall into the ventricular cavity. In a transverse section of an almost similar region of *Ichthyophis*, the same arrangement of parts is noticed by me.

The Hypothalamo-hypophysial Tract

In *Ichthyophis*, according to Gabe (1972) the neuraxons coming from the magno-cellular PON proceed posteriorly as the hypothalamo-hypophysial tract to the PN. While a ME has been described by him, any of the fibres ending in the ME is not recorded. In my figure (Pl. 2, figure 26) the tall cells of the ME (thick arrow) are clearly seen and in the external zone, blood vessels (thin arrows) are met with; these probably proceed to the PD (arrow-head). It is likely there is a portal circulation.

Hypothalamo-hypophysial system in Indian Anura

I have examined a few Indian genera (*Rana*, *Bufo*, *Ixalus*, *Rhacophorus*) and also the burrowing Microhylid narrow-

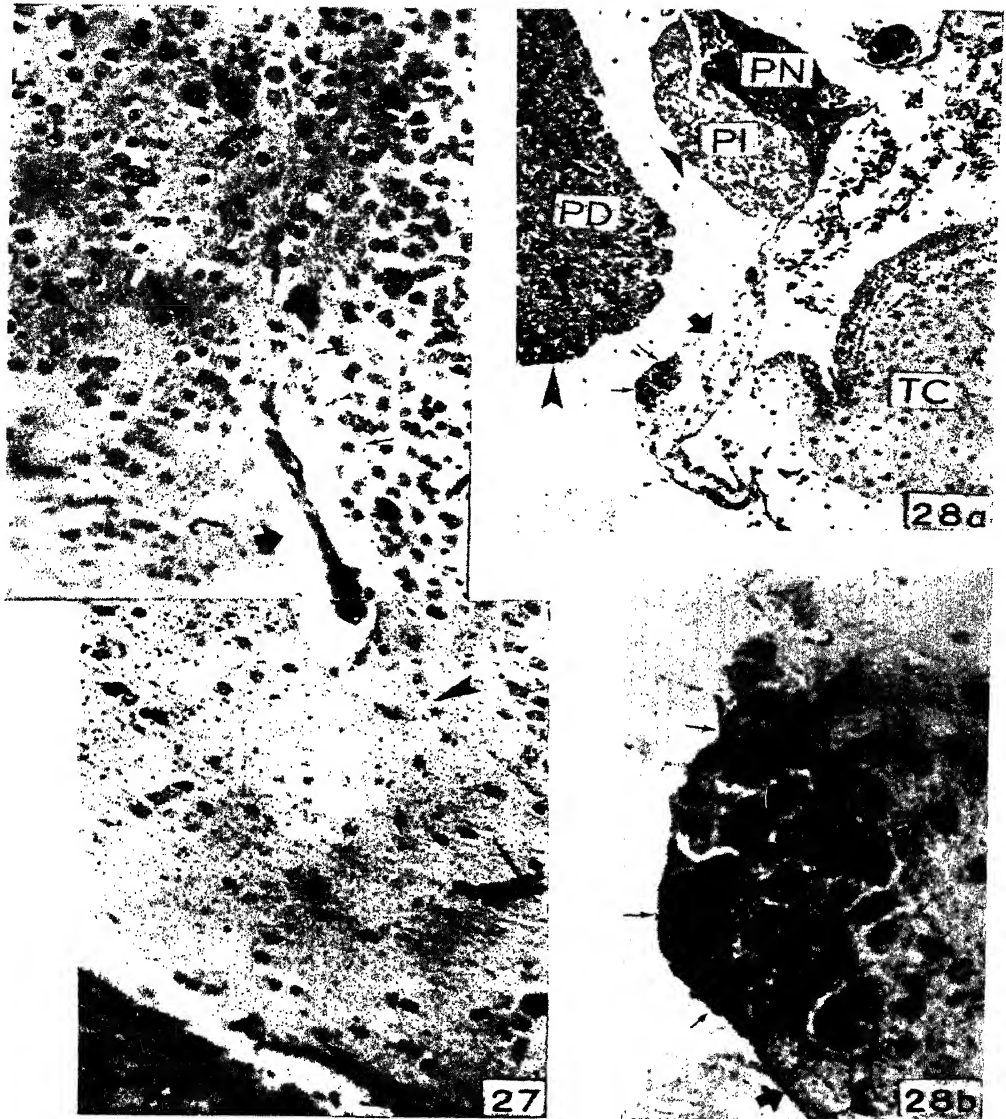


Figure 27 The PON region of *Rana tigrina* adult hypothalamic region, longitudinal section, CAH-phloxine ($\times 200$). There is one neuron (thin arrow) showing a long axon which is by the side of a blood vessel (thick arrow). Ventrolaterally to this blood vessel, there is an axon (long arrow-head) with four neurosecretory granules proceeding towards the preoptic recess (short arrow-head). In the upper part of the figure, numerous neurosecretory cells and granules can be made out

Figure 28a The pituitary gland of *Uperodon systoma*, CAH-phloxine, longitudinal section, ($\times 200$). In the median eminence (thick arrow), a group of neurosecretory cells (double arrows) can be made out and the pars distalis is in contact with this; in the picture, fixation artefact appears to have separated the pars distalis (long arrow-head) from the group of neurosecretory cells in the median eminence. The pars nervosa and pars intermedia (short arrow-head) could be made out

Figure 28b The median eminence neurosecretory cells (thin arrow) as in figure 28a, magnified, longitudinal section, CAH-phloxine, ($\times 700$). At the lower end of the picture, two degranulated neurosecretory cells could be made out (thick arrow)

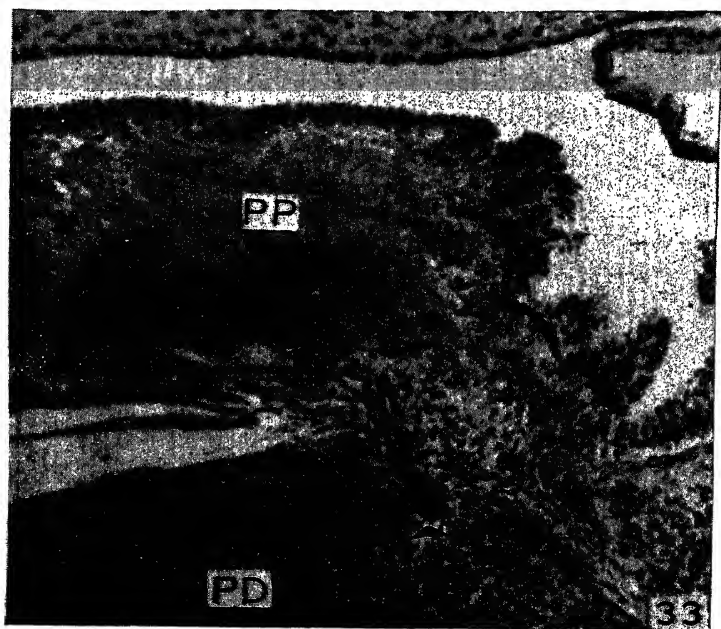


Plate 2

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Figure 26 The median eminence (thick arrow) and the pars distalis (arrow-head) of *Ichthyophis glutinosus*, CAH-phloxine, longitudinal section ($\times 200$). A part of the mesencephalic flexure is seen above the pituitary gland. The pituitary is disconnected with the infundibulum, an artefact. Blood vessels (double arrow) are seen in the median eminence which are probably portal vessels

Figure 33 The median eminence, the primary plexus (thin arrow), portal veins (arrow-head) going into the pars distalis of *Varanus*; copious neurosecretion (thick arrow) could be seen in the median eminence and in the primary plexus (thin arrow), longitudinal section, CAH-Phloxine ($\times 800$)

Figure 35 Longitudinal section of an ink-injected pituitary gland of *Calotes*, thick section, Note the vascular septum (thick arrow) between the pars nervosa and pars intermedia. The primary plexus, (large arrow-head) is superficial

mouthed toads *Uperodon systoma* and *Microhyla ornata*. In *Rana*, *Bufo*, *Ixalus* and *Rhacophorus*, the disposition of the PON is like any other frog or toad that has been described; the neurons are found in the wall of the preoptic recess; in *Rana tigrina*, some neurosecretory cells are found near the roofing epithelium of the brain; the magnocellular part of the PON is secretory. From the PON, beaded fibres can be seen proceeding towards the tuber cinerium; these probably go to the ME and/or PN. In addition to these granules, there are certain fine droplets which appear to coalesce and these as Pillay (1957) described in *Gegeneophis* are thrown into the CSF. In the examined genera listed above, there are certain neurosecretory fibres proceeding in front of the PON (Pl. 3, figure 27, thin arrow; arrow-head), (figure 29); these obviously

are the fibres which are going to form the 2nd fasciculus which later joins the posteriorly running first fasciculus.

In the Microhylids examined, the PON is noticed in the preoptic recess wall and as in *Rana*, *Bufo* etc., there are two fasciculi which join and proceed towards the PN. In *Microhyla*, I have not been able to make out the PON but axons with neurosecretion can, however, be made out. The PN and PI are vascular and there is no penetration of fibres from the former into the latter. The ME is quite large in *U. systoma*, also a Microhylid, and in it a peculiarity is noticed. There is a group of CAH positive cells (Pl. 3, figure 28a, 28b, thin arrows) towards the external layer of ME in *Uperodon* but not in *Microhyla*. While a large number of this group of cells shows stored neurosecretion, there are a few which appear to have secreted

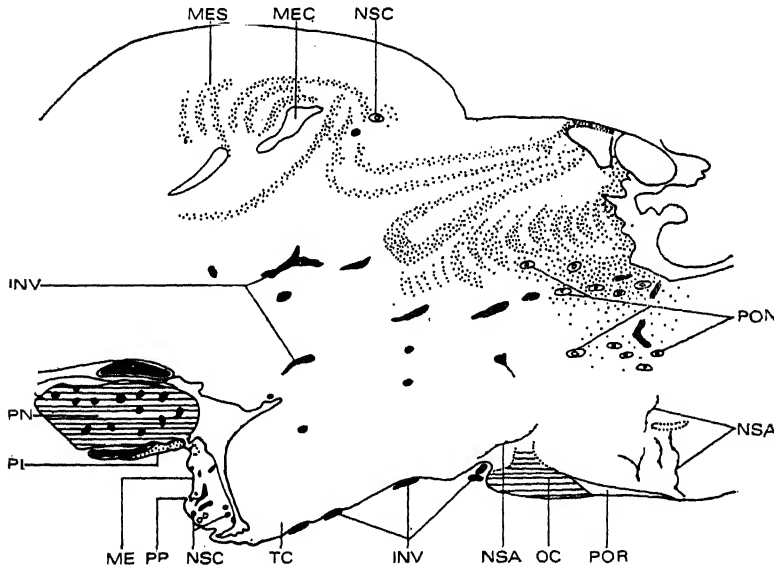


Figure 29 The hypothalamus and pituitary gland of *Uperodon systoma* collected at the Shivaji University campus. Note the neurosecretory axons in front of the preoptic recess forming the second fasciculus of the hypothalamo-neurohypophyseal tract. Note also a few neurosecretory cells in the mesencephalon. Longitudinal section, CAH-phloxine ($\times 800$)

also (figure 28b, thick arrow). Probably the secretion goes either directly or by vascular channel into the PD (figure 28a, large arrow-head). Such a concentration of neurosecretory cells in the ME of *Amphibia* has so far not been described; however, see account of *Calotes* ME. In *U. systoma*, the mesencephalon (figure 29) shows the occurrence of a few CAH positive neurosecretory cells.

Enzyme Studies

These studies have brought out interesting functional correlation between the hypothalamus and the hypophysis in *Rana esculenta* (Milone et al. 1975). Annual study of glucose-6-phosphatase, acid and alkaline phosphatases, glucose-6-phosphate dehydrogenase and β -glucuronidase has been made and the enzymes show a cyclic pattern in their activity both in the hypothalamus and in the PG. The enzyme activity reflects the hormonal changes in the reproductive organs of the frog. A very interesting relationship among the hypothalamus-hypophysis-fat body-testis of the frog has been brought out by the Naples School (Chieffi et al. 1975). Jointly a factor of the lactotrophs (A_1 cells) and/or B_2 cells (gonadotrophs) of the PD activates the fat bodies and the PG cannot activate the testis unless there are fat bodies. The authors even postulate a portal system between the fat-bodies and the testis.

Types of Neurosecretion

In the fish and frog (Srebro 1970), there are three types of neurosecretion; CAH positive granules, CAH positive globules (colloid) and CAH negative and phloxine positive colloids; the latter stain red and purple and are called neuroacidophilic colloid (NAC). The NAC is restricted to the perikaryon, while the former two are noticed in the axons. NAC is histochemi-

cally different as it contains arginine. The exact physiological role of NAC is not known. Probably it has a more metabolic role than a physiologic one.

With regard to the PON of *Amphibia*, electron microscope has revealed two types of granules in the toad nucleus (Murakami 1964). One of them ranges between 1000–3000 Å, this is fabricated in the Golgi area and is an osmophilic globule corresponding to the colloid droplets described in the light microscope.

The Paraventricular Organ

In the vertebrates, in the diencephalic region there is an organ called the paraventricular organ (figure 30) which may be an ependymo-secretory organ or may point to a receptor activity. In *Triturus punctatus* (Teichman et al. 1968), *Rana esculenta* and *Lacerta viridis*, there is no Gomori positive substance in the cells of this organ. In the newts, fibres arising from the PON and containing Gomori positive granules (neurosecretory) proceed to the paraventricular organ and passing in between the ependyma cells release the secretion into the ventricle (figure 30).

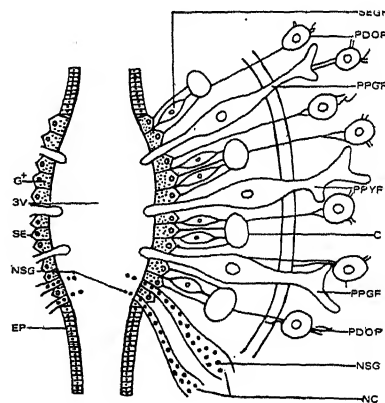


Figure 30 The paraventricular organ, diagrammatic (After Teichman et al. 1963, modified)

Development of the Median Eminence

In the Indian tadpoles studied (*Rana tigrina*), a ME could not be identified at the region of contact between the infundibulum and the PA but a vascular area at this region appears representing a ME during metamorphosis (Mathur 1969). In *Xenopus* (Srebro 1962) the ME appears at Stage 55 of the development of the tadpoles; the portal vessels appear during metamorphosis. The development of the ME marks a crucial stage in the tadpoles life-history. According to Etkin (1965), prior to the onset of metamorphosis in American tadpoles, in the contact area between the infundibular wall and the PD, the capillaries break up into a plexus. During prometamorphosis (the tadpole has developed anterior limbs also) the neural tissue in this area becomes thicker and the PD separates itself from the infundibular floor except at the anterior end where the portal vessels coming from the primary plexus in the newly formed ME enter the PD.

The ME is thyroid sensitive. Thyroidectomised tadpoles do not have it but thyroxin therapy develops it. The latter therapy fails to develop a ME in tadpoles whose adenohypophysis has been ablated (Etkin et al. 1965); thyroidectomised tadpoles also fail to metamorphose.

In the mid-larval stage of *Hyla* (Smoller 1966), the median eminence manifests itself and with the beginning of metamorphic climax, it increases in size. Neurosecretory granules are seen in the larval ME and also in the capillary plexus.

Neurosecretory granules in the Median Eminence and Pars Nervosa

In the internal zone of the ME of the bull-frog (Kobayashi & Oota 1964) (as also in the mouse, green parakeet, pigeon, turtle) there are two types of nerve end-

ings; those with neurosecretory granules and synaptic vesicles and those without neurosecretory granules but with synaptic vesicles; in the PN both non-neurosecretory and neurosecretory nerve endings are met with. Among the non-neurosecretory axons, processes of the ependymal and glial cells are also seen; some of these processes terminate on axons and their functional relationship is not known. The neurosecretory axon endings in the ME or the PN do not show endoplasmic reticulum or Golgi bodies. Probably the fabrication of the neurosecretory material is in the perikaryon and its maturation in the axon. The neurosecretory granules in the ME vary in size from those in the PN; the neurohypophysial hormones may be different, therefore, in the two areas; it is likely that the smaller granules of the ME and PN contain arginine vasotocin and the larger granules of the PN contain oxytocin (Kobayashi & Oota 1964).

Neurosecretion in Reptiles

The Hypothalamo-hypophyseal system in Reptiles

In the previous groups, reference was made to a PON whose neuraxons formed the hypothalamo-hypophyseal tract. In the groups that follow,—the amniota, there are two nuclei (on either side) whose neuraxons help form the hypothalamo-hypophyseal tract. They are the SON and PVN. The SON alone or along with the PVN is considered to be the homologue of the PON of the fish and Amphibia.

In the reptiles, the SON is noticed in two parts: in a longitudinal sectional view of the brain, a darkly staining group is noticed at the anterior region of the optic chiasma and a similar posterior group at the posterior region; bridging neurosecretory cells are present between the two

groups. The PVN is situated in between these two dorsally in the hypothalamus and extend obliquely.

In the snake *Thamnophis*, Scharrer (1951) described a few fibres proceeding on either side of the 3rd ventricle from the PVN to the paraphysis. Anantanarayanan (1955) after examining the Indian species *Calotes*, *Sitana* (Lacertilia), *Tropidonotus* (Ophidia) and *Lissemys* (Chelonina) came to the conclusion that the neurosecretory tract from the PVN to the paraphysis is noticed in all these and therefore, it seems to be a common feature of the reptiles.

The hypothalamic nuclei (both PAS positive and negative) have been examined in detail by Prasada Rao and Subhedar (1977) in the Indian garden lizard *Calotes*. I have also examined *Calotes* with regard to its portal circulation and neurosecretion; I am able to confirm their findings as regards the SON and PVN. The SON shows the ventral, lateral, medial and entopeduncular subdivisions as stated by them; there are less intensely stained CAH positive cells bridging the SON cells with PV cells. Unfortunately they have not described the peptidergic fibre tracts arising from these and going to the PN. In my preparations of the longitudinal sections of the brain of *Calotes* (figure 31) and *Varanus* (figure 32) stained with CAH-phloxine, beaded fibres from the PVN towards the SON and similar ones from the SON to the PN passing through the ME are clearly made out. This is the peptidergic tract. I could not however, make out in my preparations any varicose fibres proceeding from the PVN towards the paraphysis as described by Anantanarayanan (1955) in *Calotes*, *Sitana*, *Tropidonotus* and *Lissemys* also shown in his diagrammatic figure 3. Gesell and Callard (1972) also could not find a hypothalamo-paraphysial tract in the lizard *Dipsosaurus* examined by them, supporting Pandalai's (1966) general statement

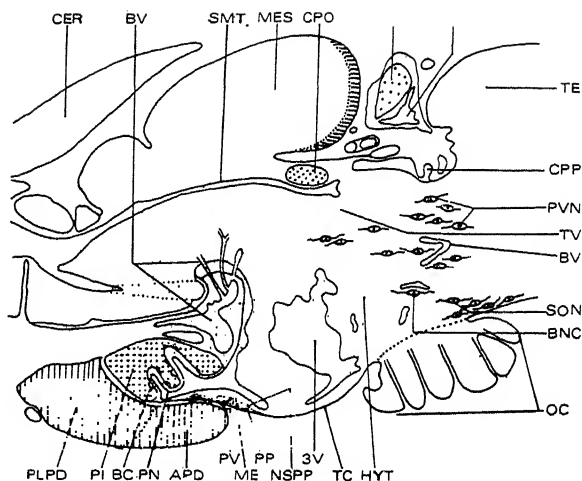


Figure 31 The hypothalamus and pituitary gland of the Indian garden lizard, *Calotes versicolor*, longitudinal section, CAH-phloxine ($\times 800$). Note the neurosecretory granules in the median eminence. The hypothalamo-neurohypophysial tract is not visible in the other regions. The primary plexus is superficial. Bridging neurons between the SON and PVN are seen. A hypothalamo-paraphysial tract is not seen. The posterior part of the pars distalis is phloxine positive.

that no extrahypothalamic neurosecretory cells exist in the lizards. The above authors (Gesell & Callard 1972), however, describe a separate paraventricular-pars nervosa tract in *Dipsosaurus*. Such a tract I have not noticed in *Calotes* or *Varanus* studied by me.

Both in *Calotes* and *Varanus* (figure 31, 32), as in *Dipsosaurus* (Gesell & Callard 1972) quite a few fibres of the peptidergic tract become associated with the portal capillaries and release the CAH positive granules near the primary plexus (Pl. 2, figure 33); this neurosecretion probably goes to the PD through the portal veins. More than this, and particularly in *Varanus* (figure 32) CAH positive granules from the hypothalamo-neurohypophysial tract become closely associated with the ependyma and may get into the 3rd ventricle.

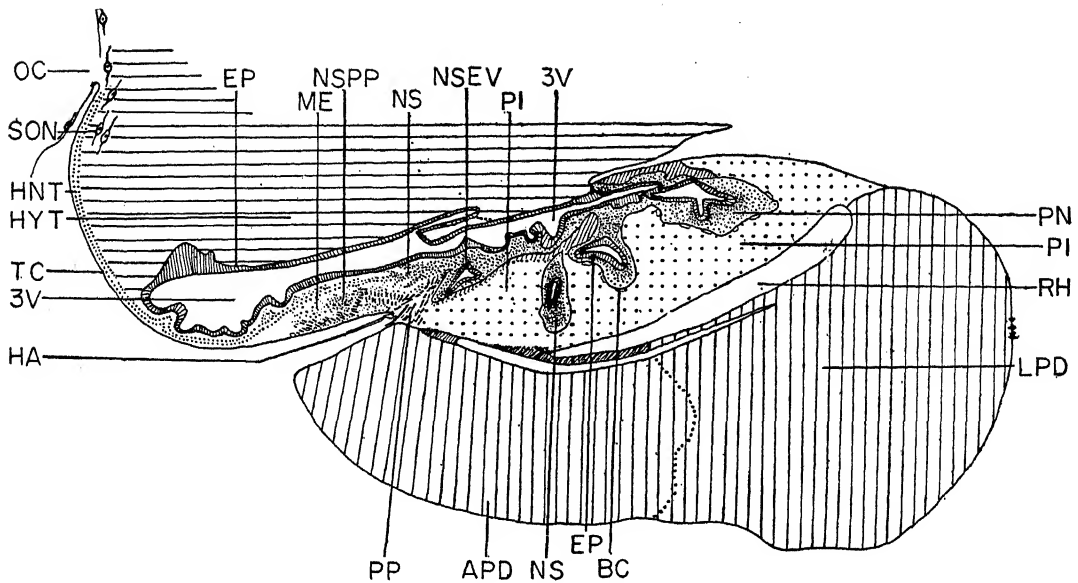


Figure 32 The hypothalamus and pituitary gland of *Varanus*, longitudinal section, CAH-phloxine ($\times 800$). Note the lobes of the pars nervosa loaded with neurosecretory granules; the neurosecretion is also noticed to enter the portal circulation and the 3rd ventricle. The hypothalamo-neurohypophyseal tract is faintly visible coming from the SON; in the median eminence the secretory granules are stained dark. The posterior part of the pars distalis is phloxine positive

In certain regions, the ependymal cells appear to make way for the secretion to pass into the ventricle (figure 32, NSEV). However, in all these situations, both in *Calotes* and *Varanus*, the neurosecretory granules have never been seen by me in the ventricular cavity.

In *Varanus*, the hypothalamo-neurohypophyseal tract could be easily followed from the SON to the PN (figure 32); in *Calotes*, the tract is seen only in the region of the ME (figure 31).

In the krait (*Bungarus*) studied by me, transverse sections of the diencephalon region of the brain disclose the peptidergic tract in the ME; laterally the extension of the SON could also be made out and the neurosecretory granules are particularly large. In the region of the subcommissural organ, the paraventricular organ is

met with; a few bipolar PV nuclear neurons are associated with this.

In the marine snake *Enhydrina valakadien* (figure 34), the hypothalamo-neurohypophyseal tract starts from the optic chiasma and proceeds to the PN through the ME. Unlike *Bungarus*, the neurosecretion is in the form of colloidal globules; many of them find their way into the 3rd ventricle; a few are noticed in the hypothalamic region also. In the PN, the secretion is stored in the form of granules. In the tract, secretory granules are not present. More mesially the PVN can be made out and it is not clear how the neurosecretion from this reaches the PN. The PI is posterior to the solid PN and is independent of the latter. The PD is close to the median eminence anteriorly and the portal veins pass through a con-

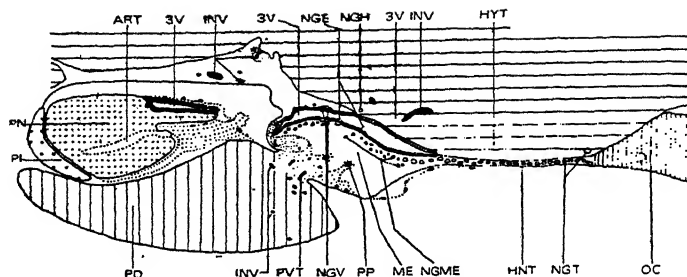


Figure 34 The hypothalamus and pituitary gland of the sea-snake *Enhydrina valakadien*, longitudinal section, CAH-phloxine, ($\times 800$). From the SON, the hypothalamo-neurohypophyseal tract starts and has colloidal globules which go to the solid pars nervosa; in the pars nervosa only CAH positive granules are seen. A number of CAH positive globules can be seen in the 3rd ventricle

nective tissue bridge called pars terminalis into the PD; in India ink injected specimens, the primary plexus and the portal veins can be easily made out. The posterior part of the PD is phloxinophilic as in *Calotes* and *Varanus*; the anterior part has in addition to basophilic cells, a few acidophils also. In the sea-snake, in a ventral view of the brain, the PD completely covers the PN and PI.

I have not been able to make out a hypothalamo-paraphyseal tract in the krait or in the sea-snake.

In the ME of *Calotes* (Prasada Rao & Subhedar 1977) the presence of rare AF positive neurons are described; I have not noticed such neurons in *Calotes* or in *Varanus*. In *Calotes*, individual variations are quite common.

In the chelonian *Lissemys vittata* studied, the ME shows a PT ventrally in close contact with it. The portal veins make their way through the PT into the PD. The PT sends follicular cords into the ME and these open into the 3rd ventricle. The hypothalamo-neurohypophyseal tract could not be made out even though the animal was a gravid one. Neurosecretory granules could be made out in abundance only in the PN and the interdigitation of the

PI and PN gives an impression that the posterior lobe hormones affect the physiology of the intermediate lobe. Some follicles of the intermediate lobe contain phloxine positive colloid in the lumen giving a spurious appearance of a thyroid follicle.

With regard to the PG in reptiles, Saint Girons (1970) reported that the PI may be atrophied in some lizards (Amphisbaenians) or absent in some snakes (*Typhlops*, *Leptotyphlops*) and he came to the conclusion that the volume of PI depended on the light that these animals received. The PI is hypertrophied in *Chamaelion* and other tropical lizards.

The intermediate lobe of reptiles as also of other vertebrates except Amphibia, is innervated with small granule (700 Å fibres which cannot be stained with the usual stains colouring the neurosecretory fibres; in all vertebrates with the exception of Amphibia, a set of large granule (1000—3000 Å fibres stainable with the typical neurosecretory dyes are also present. Both these fibres make synaptic contacts with the cells of the PI. Knowles (1963) considered in the dogfish that the small granule fibres were inhibitory while the large granule fibres control the synthetic mechanism. Later research has not lent support

to this; the large granule fibres are absent in Amphibia. As pointed out in the Amphibian section, there may be an intimate contact, nay even fusion in certain regions between the partes intermedia and nervosa to which Iturriza (1964) and Rodriguez (1971) also refer.

The hypothalamo-hypophysial axis of the lizard *Klauberina* (Rodriguez 1971) shows a primitive tetrapod condition. The infundibular recess is deep; there appears to be no pituicytes in the ependyma. Particles from the CSF pass through the ependyma into the basement membrane. A peculiarity of great interest is the presence of a vascular septum between the PI and PN. Vascular processes from the septum enter the PI. These probably bring in the secretion from the PN and there are no fibres in the PI.

Sheela and Pandalai (1965/66) in describing the angioarchitecture of the PG of *Calotes* state that a special arterial branch (see their figure 2) from the infundibular artery supplies blood to PN; this forms a vascular septum as in *Klauberina* (Rodriguez 1971) between the two layers. Vascular projections are noticed into the PN and the neuraxons coming from the SON and PVN end near these projections. There are also highly branching vascular projections into the PI and the neurosecretion thus appears to pass into the PI also. The authors state that as *Calotes* is capable of changing colour, it has a well developed intermediate region. According to Malcolm A. Smith (Sauria, Fauna of British India), it is only the male *Calotes* that is capable of changing colour generally while fighting (agonistic). It is not clear in what way the posterior lobe hormones influence the intermediate cells.

I have examined the sectional views of the PG of *Calotes* and *Varanus* during the non-breeding season. In the injected specimens, I have not noticed a special arte-

rial branch supplying the PN of *Calotes* as described by Sheela and Pandalai (1965/66). However, in thick sections, the formation of a vascular septum (Pl. 2, figure 35) between the PN and PI is noticed in the garden lizard. The profuse distribution of vascular branches into the PI and PN as shown by Sheela and Pandalai is not seen. In *Varanus* the PI shows better organization than in *Calotes*; surrounding the lobules of the PN there are capillaries and these do not seem to send highly branching ones into the PI; blood capillaries are present between the lobules of the PI in *Varanus*. In both the examples studied by me, from the ME there are portal veins going into the anterior part of the PD which is largely basophilic.

Jacob (1965) examined the hypothalamo-hypophysial relation in some Indian reptiles and noted as follows: The infundibular recess extending into the PN may be long or short; when short as in the snakes *Eryx* and *Ptyas* and the chelonian *Lissemys*, the PN appears solid. Only in *Varanus*, the PN showed a folded nature. In a longitudinal sectional view of the brain of *Varanus* (Ariens Kappers et al. 1936—65), the different parts of the diencephalon, the anterior and posterior palli and the posterior commissure are depicted. A non-glandular PT (Jacob 1965) is described in between the ME and PD through which the portal veins pass into the PD in *Varanus*, *Uromastix*, *Eryx* and *Ptyas*. In *Lissemys* (Jacob 1965), the Japanese chelonian *Clemmys* (Hirano 1966) and the crocodile (Nemec 1952, Jacob 1965, and Saint Girons 1970) a pars terminalis is described. In *Crocodylus niloticus* (Gabe & Rancurel 1958), the SO and PV neurosecretory cells are small as in other reptiles. The PN is also small and is surrounded by the PI. Fibres are noticed to carry neurosecretion from the PN into PI, also seen in *Vipera* and *Natrix* Bargmann et al. 1950)

and *Natrix* (Bargmann et al. 1950).

The Portal Circulation

Enemar (1960) gives a figure of the portal circulation in *Lacerta* which is reproduced here (figure 36) considerably simplified. A branch from the internal carotid artery, called infundibular artery divides into anterior and posterior rami. The anterior ramus distributes itself over the infundibular region. The posterior, after giving off branches to the infundibulum, also irrigates the ME. A primary plexus at the ME is formed from capillaries covering the infundibular floor. The primary plexus appears to extend over the anterior part of the PI also in *Lacerta*. Two portal veins from the primary plexus proceed to the anterior part of the PD.

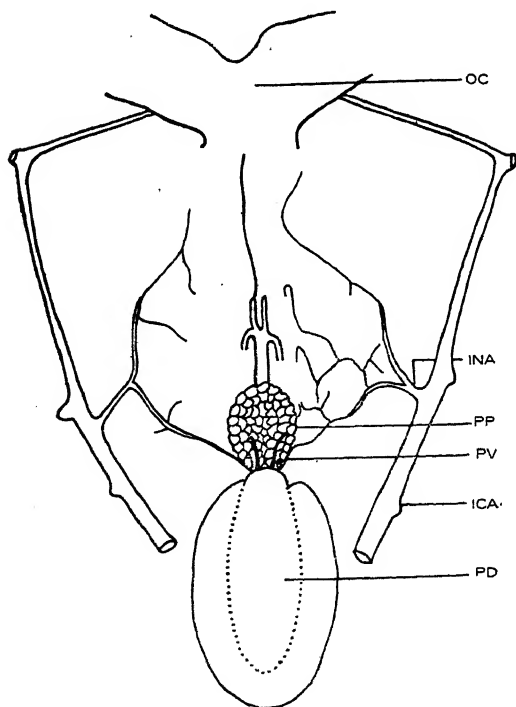


Figure 36 The ventral aspect of the infundibulum and pituitary gland of *Lacerta* showing the primary plexus, diagrammatic (After Enemar 1960, modified)

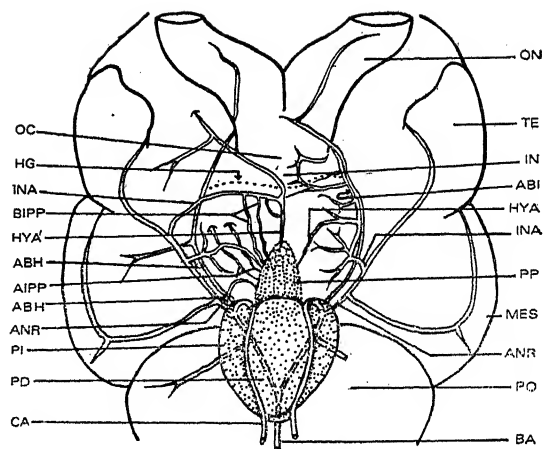


Figure 37 The ventral aspect of the brain in the infundibular region showing the pituitary gland of *Calotes*. The primary plexus formed by hypophyseal and other arteries could be made out; it extends over the pars intermedia also. Vessels are not symmetrically disposed on either side. Ink-injected specimen

I have examined the angioarchitecture of the PG of *Calotes* (figure 37), *Varanus* (Lacertilia) and *Tropidonotus* (figure 38) and *Enhydryna* (figure 39) (Ophidia) by injecting the artery. In the lizards, the infundibular artery (figure 37), arising from the internal carotid artery gives off a branch—the hypophyseal artery which proceeds towards the hypophysis. The primary plexus appears to be formed by these arteries and the plexus extends over the intermediate lobe also. Portal veins from the primary plexus extend over the PD and they form the secondary plexus. In the snakes examined (figures 38, 39), the infundibular artery gives off a hypophyseal artery which proceeds towards the hypophysis; in *Tropidonotus* (figure 38) on one side, the hypophyseal artery appears to proceed to the posterior region of the PD. The primary plexus so clearly seen on the ventral aspect of the infundibulum in the lizards is not met with in the snakes.

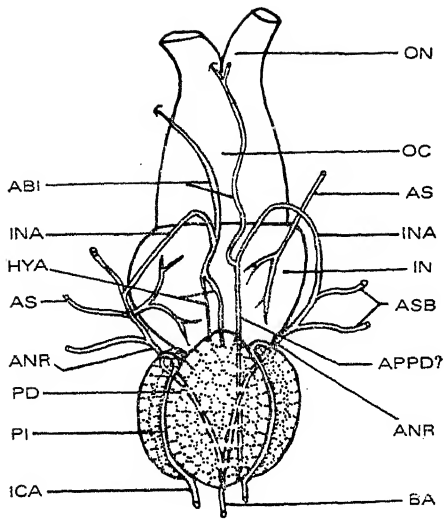


Figure 38 The ventral aspect of the infundibulum and pituitary gland of the fresh-water snake *Tropidonotus piscator*. India ink injected specimen. The primary plexus is not seen here as in *Calotes*. Peculiarly there appears to be a vessel going to the posterior part of the pars distalis on the left of the snake

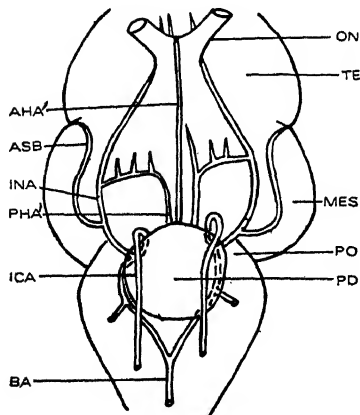


Figure 39 Ventral aspect of the brain and pituitary gland of the sea-snake *Enhydrina*. The pars nervosa and pars intermedia are covered over by the pars distalis (see figure 34). The primary plexus is deep

Wingstrand (1966) who made a comparative study of the reptilian PG came to the following conclusions to which information about the ME and portal circulation has now been added:

1. The neural lobe may be folded (*Sphenodon*, *Varanus*, *Alligator*, some chelonians) or single (*Uromastix*, crocodile, *Caiman*, *Lissemys*) or compact (snakes). It becomes massive in the burrowing lizards and amphisbaenians as in the *Ophidia* (Saint Girons 1970).
2. Morphologically, the ME is uniformly single in all reptiles; in *Lissemys*, it appears double. Kobayashi and Wada (1973) described double ME in reptiles and birds. In *Lepotyphlops*, the ME is hypertrophied and is larger than PN (Saint Girons 1970).
3. A PT develops in all reptiles except in *Lacertilia*, *Ophidia* and crocodile where a nonglandular PT is seen through which the portal vessels pass into the PD.
4. A hypophysial cleft is present in *Sphenodon*, *Varanus* and *Lacerta* (of the examined lizards), *Ophidia* and *Testudo* (of the examined Chelonians) and is absent in the others.
5. A PI is developed; see, however, Saint Girons (1970) for exceptions.

Neurosecretion in Aves

The Hypothalamo-Hypophysial System in Aves

The avian hypothalamus shows certain peculiarities. They are as follows:

1. The neurosecretory cells are not collected into nuclei as in mammals but are in diffuse clusters which are highly vascularised. Some optic fibres may also end in the SON.

2. The portal capillaries are very superficial (figure 40) on the ventral aspect of the ME.
3. The hypothalamus shows two MEs (Farner & Oksche 1962, Matsui 1966, Assenmacher & Calas 1969), the anterior of which is AF positive. Two sets of portal vessels (Vitums et al. 1965) anterior and posterior, drain the media eminentia and enter the cranial (rostral) and caudal (proximal) parts of the adenohypophysis respectively; these two parts show two different types of acidophils. An intermediate lobe is absent. In some birds like *Melopsittacus* (Kobayashi et al. 1961), however, the PG does not show double lobes and the portal veins are also not divided into two sets.
4. *The fibre tracts:* Workers in this field have uniformly said that it is difficult to trace the tracts in birds. Wingstrand (1951) described four tracts reaching the neurohypophysis in birds: they are (1) anterior hypophysial, (2) the supraoptico-hypophysial, (3) the tubero-hypophysial, and (4) the posterior hypophysial tracts. The fibres from the lateral and inferior nuclei send the anterior hypophysial tract. The SON sends the supraoptico-hypophysial tract. The tuberal nucleus sends the tubero-hypophysial tract which may end up in the ME. The posterior hypophysial tract arises from a nucleus situated ventrally to the decussation of the infundibular tract.

In his original figure of the hypothalamic-hypophysial tract of *Zonotrichia* Oksche (see Greep 1963, figure 31) showed the magnocellular-hypophysial tract coming from the SON and PVN terminating in the PN; from some of

these axonic fibres, collaterals arose and they ended up near the superficial portal capillaries of the anterior ME. The fibres from SON and PVN send their fibres (hypothalamo-hypophysial tract, figure 40) to the neural lobe (Oksche et al. 1971). Non-fluorescent Gomori positive neurons from the anterior hypothalamus send their axons to the anterior ME; a few of these fibres may also go to the posterior ME. Non-fluorescent Gomori negative neurons from the anterior and posterior hypothalamus go to anterior and posterior MEs respectively. This was the tubero-infundibular or parvicellular hypophysial tract coming from the infundibular and ventromedial nuclei (Greep 1963). They have also now described fluorescent tuberal neurons forming the aminergic system sending fibres to the portal vessels in the anterior and posterior MEs.

In Scharrer's diagrammatic figure reproduced here (figure 41), the ME is single and more importantly, some fibres coming from the hypothalamic nuclei go directly to the PN while others coming particu-

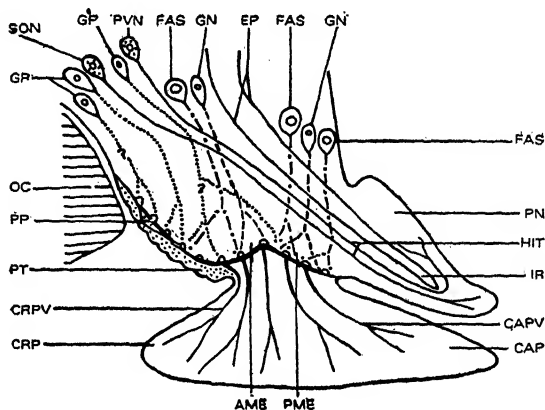


Figure 40 The hypothalamus and the pituitary gland of the bird *Zonotrichia*, diagrammatic (After Oksche et al. 1971, modified) The median eminence, portal veins and the pituitary gland are double in many birds

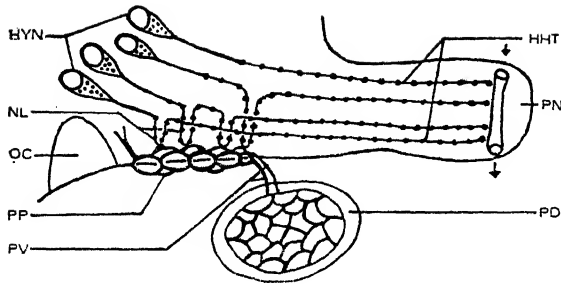


Figure 41 The hypothalamus and portal circulation of a bird, diagrammatic (After Scharer 1953, modified) Note the superficial primary plexus

larly from NLT of the hypothalamus send loops to the superficial portal circulation before reaching the PN; this has to be revised in the light of the above description.

In *Columba*, Matsui (1966) has not shown in his figure (figure 20), the neurosecretory nuclei; however, in the anterior ME, large number of fibre terminals show large AF-positive granules which are probably neurosecretory in nature with synaptic vesicles. There are a few fibre terminals with smaller granules with synaptic vesicles. In the posterior ME, few fibre terminals with large neurosecretory granules (90–120 m μ and less electron dense, about 10%) and synaptic vesicles, and a large number of terminals with smaller granules which are probably monoamines (70–150 m μ and a little more electron dense, about 30–40%) and synaptic vesicles and few terminals with only synaptic vesicles. The monoamine-carrying fibres arise from the nucleus tuberis and possibly these are 'adrenergic' (Bern & Nishioka 1965).

In the duck (Assenmocher & Calas 1969), the rostral or anterior ME shows more of fibres having numerous dense granules ($\approx 1200 \text{ \AA}$) and therefore mainly neurosecretory while the caudal zone has granules ($< 100 \text{ \AA}$) which are monoamines. There are also fibre terminals show-

ing only synaptic vesicles. The nerve endings mix up with the feet of the ependymocytes giving rise to the palisade layer. The rostral parenchymal basement membrane appears very fine and rectilinear while the caudal part shows many digitations and invaginations penetrating deeply into the ME.

The double nature of the ME, of the portal system and of the pituitary has been described in Indian birds (Singh & Dominic 1972). Quite a few birds have been known to have a single portal vessel (see discussion, Oksche 1971), p. 907). Ghosh and Ghosh (1972) have studied the effect of stress on the hypothalamic nuclei of birds. This is particularly significant as birds lack a PI and it is known that animals with a well developed PI resist dehydration efficiently. Water deprivation in the arid-zone bird *Lonchura* caused enlargement of the nuclei and nucleoli of the neurons of the SON.

The Structure of the Median Eminence

The five layers (figure 42) of the two zones of the avian ME (Kobayashi & Ishii 1968) (also seen in other groups of vertebrates with some modifications) and the histology of the different layers of the same (Kobayashi et al. 1970) have been documented. It is given in table 1.

With regard to the ependymal cells, Matsui and Kobayashi (1968) describe at least three types of projections into the CSF of the rat and white crowned sparrow; they are bleb-like or finger-like microvilli or surface folds. These help in the exchange of fluids between the CSF and blood in the primary plexus. This is possibly under the control of monoaminergic neurons.

Limitations of the Median Eminence

Externally it is limited by the capillaries that vascularise the basal area of the hypo-

Table 1

| | | |
|---------------|------------------|--|
| Internal zone | Ependymal layer | Ependymal cells |
| | Hypendymal layer | Hypendymal & glia cells, ependymal processes |
| | Fibre layer | Supraoptico-hypophyseal tract; glia cells; ependymal, hypendymal and glia processes |
| External zone | Reticular layer | Supraoptico-hypophyseal tract; tubero-hypophyseal tract or infundibulo-hypophyseal tract; glia cells; ependymal, hypendymal and glia processes |
| | Palisade layer | same as reticular layer |

thalamus and which drain into the PD; internally by the secretory ependyma whose processes terminate at the capillaries referred to above. The distal parts of the nerve fibres of Gomori positive and Gomori negative neurons and glial cells within this area are also parts of the ME.

The neuronal perikarya in this area and the PT are excluded from this area. In fishes, the NLT is excluded from this area. According to Kobayashi et al. (1970) the ME which appears to be a specialised area is found in all vertebrates except cyclostomes. Previous workers have also pointed out (Follenius 1965, Hill & Henders on 1968, Henderson 1969), that in teleosts (where the presence of a ME has not been accepted) the posterior portion containing Gomori positive material would correspond with the PN and the anterior one to the ME.

Four types of fibres are identified by their inclusions in the ME of birds (Matsui 1969):

1. 1500—1800 Å in diameter + synaptic vesicles (500 Å) = neurosecretory
2. 1000—1200 Å in diameter + synaptic vesicles (500 Å)
3. 800—1000 Å in diameter + synaptic vesicles (500 Å) = monoaminergic
4. 500 Å only synaptic vesicles = cholinergic

Fine Structure of the Median Eminence

The ependymal cells covering the ME may exist in two forms: small and flat or large and cylindrical (Kobayashi et al. 1970). Bleb-like microvilli or bulbous protrusions suggest ependymal secretion into the 3rd ventricle; finger-like microvilli and marginal and surface folds probably absorb from the CSF. The entire cytoplasm and nucleus may be thrown into the CSF.

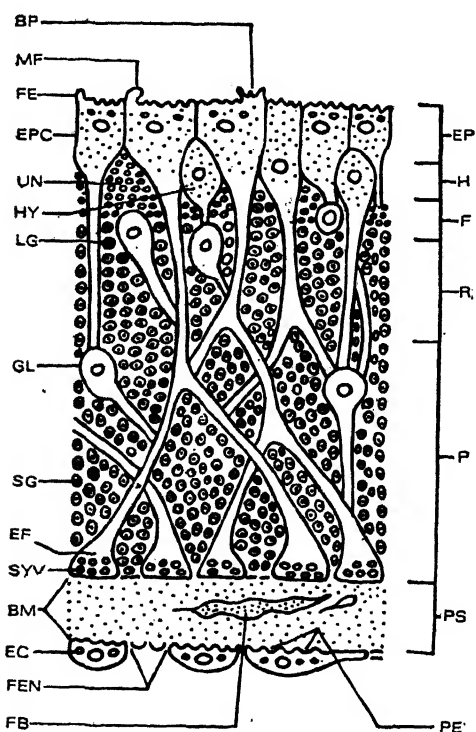


Figure 42 The avian median eminence, EM study, diagrammatic (After Kobayashi and Ishii 1968, modified)

Synaptoid contact between axons and ependymal or glial processes are seen; such contacts between axons and hypendymal or glial processes are also seen in the pigeon (Kobayashi et al. 1970) or the rat ME.

After examining the ME of birds and mammals (including monkeys) Duvernoy (1972) pointed out that the ventricular fluid may establish direct contact with the portal vessels by the partial disappearance of the ependymal layer, or that ependymal cells may group themselves round subependymal plexus which may come in contact with the surface network. Fibre terminals of the tubero-infundibular neurosecretory fibres coming from the neurons in the arcuate nucleus palisade in the external zone of the ME. This region contains transmitters, dopamine and possibly nor-epinephrine also. The nerve terminals also show clear synaptic vesicles and dense core vesicles. Acetylcholine may not be present in these synaptic vesicles as choline esterase content of the ME is very low (Clementi & Ceccarelli 1970). Recent researches have shown that the hypothalamic nuclei show choline acetylase; the latter is a better marker than acetylcholine esterase; the ME exhibited a high choline acetylase activity.

Neurosecretion in Mammals

The Hypothalamo-Hypophysial System in Mammals

A part of the diencephalon, the hypothalamus could be divided into the anterior, medial and posterior parts in an antero-posterior direction; from side to side, a lateral and a medial portion could also be made out. From above downwards, the anterior hypothalamus contains the anterior nucleus and the suprachiasmatic nucleus (Clementi & Ceccarelli 1970). The neurons here are oestrogen sensitive and control the cyclic release of FSH and LH.

These neurons may also contain significant quantity of LHRH and they may also regulate thyrotropin release (Clementi & Ceccarelli 1970).

The medial hypothalamus contains the ventromedial nucleus (Clementi & Ceccarelli 1970). There are two types of cells; large ones with dense cytoplasm and the small ones contain pale cytoplasm. A part of the ventromedial nucleus controls the release of FSH. The neurons also seem to control GH secretion; they are sensitive to glucose and there is an intimate connexion between glucose metabolism and GH secretion.

The ventromedial nucleus may send its dendrites into the lateral hypothalamus but this is not supported by the Hungarian School (see Szentagothai et al. 1968). Cross (1965) assumed that fibres from the lateral hypothalamus would make the arcuate nucleus release tonic FSH and LH by sending suitable impulses. However, Halász and Gorski (1967) showed in rats that when the hypophysiotropic area is isolated from the lateral hypothalamus area, they did ovulate.

The posterior hypothalamus (Clementi & Ceccarelli 1970) contains the arcuate nucleus which is situated in the periventricular region below the ependymal cells of the 3rd ventricle. This nucleus along with the ventromedial nucleus appears to control basal or tonic secretion of LH. The arcuate nucleus is also responsible for FSHRH secretion. Some neurons are also dopaminergic. Dopaminergic fibres of the tubero-infundibular system start from the arcuate nucleus and end near the portal blood vessels of the ME. Some axon endings contain clear synaptic vesicles and others may contain clear synaptic vesicles and dense core granules.

The anterior, ventromedial and arcuate nuclei and categorised as parvicellular nuclei while the SON and PVN are termed

magnocellular nuclei. The ME of the hypothalamus has been identified as an important co-ordinator in transmitting neurosecretion through the portal system into the adenohypophysis. A reference has also been made to the tanycyte or branching ependyma cells in bringing endocrine material from CSF into the portal system. In addition to these modes, there are other circumventricular specialised areas which help in the integration of blood, brain and the CSF. These are the organum vasculosum lamina terminalis, the subfornical organ, the pineal, the sub-commissural organ, the area postrema, the neural gland and including the ME, these get to be called (metaphorically) the seven windows of the brain (Knigge 1975).

The Hypothalamic Nuclei

For the sake of convenience, all the nuclei (accumulation of nerve cells or neurons) of the hypothalamus may be projected in a single figure (Flerkø 1970). Starting from the anterior region, we have the pre-optic area (figure 43), suprachiasmatic, supraoptic, anterior hypothalamic and paraventricular nuclei; in the medial region; we have ventromedial nucleus and in the poste-

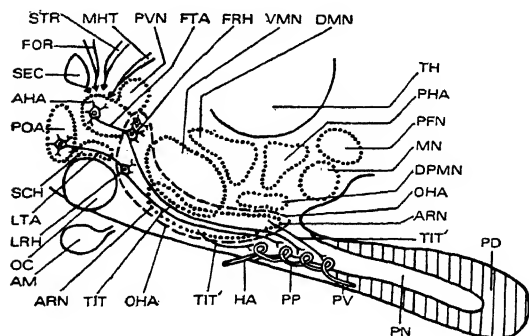


Figure 43 The mammalian hypothalamus and pituitary gland showing the hypophysiotropic area and the triggering mechanism for release of gonadotropins, diagrammatic (After Flerkø 1970, modified)

rior, there is the arcuate nucleus. Also the dorsomedian, posterior hypothalamic, dorsal premammillary, ventral premammillary and the mammillary nuclei are seen.

The Hypothalamo-Adenohypophysial System

The Hypophysiotropic area (HTA): Way back in 1966, McCann and Dhariwal, put forward that the SON and PVN and possibly other nuclei of the hypothalamus secrete certain specific neurohormones or neurotransmitters that are responsible for the activity of the adenohypophysis. This meant that the nuclei in addition to synthesizing the posterior lobe hormones (oxytocin, vasopressin) which made their way into it, the same axons also carried the neurotransmitters which comprised the releasing and inhibiting hormones and fibres from the supraoptic-paraventricular-neurohypophysial axons reached the portal vessels which carried these chemicals to the adenohypophysis. Motta et al. (1970) lent support to this view when they said (p. 472) that some extrahypothalamic neural stimulus reaches the PVN and causes the synthesis of FSHRH and this reaches the PG through the portal vessels. While that may be so in birds, in mammals, from the magnocellular complex (SON, PVN) the neurosecretory axons start and pass through the ME into the PN where they terminate at the capillaries; the neural lobe, therefore, is also a neurohaemal organ like the ME as already indicated. The tract should be called magnocellular-neurohypophysial tract carrying oxytocin and vasopressin synthesized in the SON and PVN. This would correspond to the 'posterior lobe system' of previous workers referred to by Christ (1966). Then where are the hypophysiotropins or neurotransmitters synthesized and how are these carried to the anterior lobe of the

PG return to synthesize and release the tropic hormones FSH, LH, ACTH, TSH, GH and prolactin and possibly lipotropin? In order to know this, we must know something about the 'hypophysiotropic area' demarcated in the hypothalamus (figure 44) by a series of elegant pituitary transplantations into the area and noting the effect on the basophils by the Hungarian School (Halász et al. 1962). It has already been described that the hypophysiotropic area comprises the following nuclei: the anterior periventricular nucleus, arcuate nucleus, premammillary and medial halves of the ventromedial nuclei (Flerkó 1970). It is from this area that the tubero-infundibular tract (parvicellular-infundibular tract) arises and goes to the ME. The fibres terminate at the capillary loops which later on return to the hypothalamo-hypophysial portal vessels. The nerve cells present in the tubero-infundibular tract are supposed to synthesize releasing hormones (RHs) and inhibit-

ing hormones (IHs). A question arises if all the RHs and IHs are produced in the limited hypophysiotropic area? Lesion experiments (Mess et al. 1966) in the hypophysiotropic area while pointing to the existence of FSHRH and LHRH areas, nothing could be known about other hormones. FSHRH is synthesized in the paraventricular region and LHRH in the suprachiasmatic and arcuate ventromedial regions. Tima (1971) stated that the TSHRH and other releasing hormones are widely distributed in the hypothalamus. Later researches have shown that the suprachiasmatic, paraventricular and anterior hypothalamic nuclei may also show thyrotropic areas (Flament-Durand & Desclin 1970). Mess et al. (1970) include the suprachiasmatic, paraventricular, periventricular, anterior hypothalamic, arcuate and premammillary and medial halves of the ventromedial nuclei in the hypophysiotropic area and these generate the RHs and IHs. Thus the original configuration of the hypophysiotropic area has been enlarged now. According to the same authors, all the releasing hormones may be stored in the ME and released as the occasion demands. The ME therefore, forms an anatomical interface between the brain and the PG.

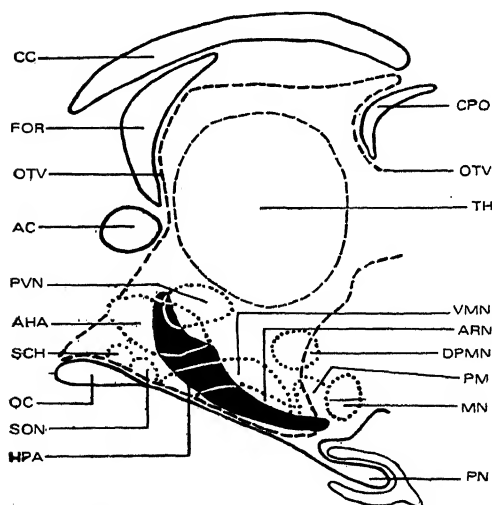


Figure 44 The mammalian hypothalamus showing the hypophysiotropic area (HPA) and other nuclei, diagrammatic (After Halász et al. 1962, modified)

The Hypophysiotropic Hormones or Factors

Of historic importance, it was in 1955 that Saffran et al. introduced the term 'releasing factors' to describe the corticotropin releasing factor (CRF) which releases ACTH. At the same time, Guillemin used the term 'Hypophysiotropic factor'. Actually the term 'hypophysiotropic' was introduced by Guillemin and Rosenberg (1955) to connote the supportive or 'nutritive' effects of the chemical (hypophysiotropin) on the explanted or transplanted adenohypophysial parts. Meites (1966) in-

roduced the term 'inhibiting factor' for describing Prolactin inhibiting factor (PIF) and growth hormone inhibiting factor (GIF). In 1967, Schally used the term 'hypothalamic releasing factors'.

Sites of Synthesis of Releasing and Inhibiting Hormones

There is considerable difference of opinion with regard to the sites of synthesis of the above hormones or factors. The studies have been conducted mostly on rat but dog, cat, sheep, rabbit and guinea pig also have been tested. These studies were made during 1961—68.

CRH has been noticed in the stalk median eminence (SME) and SON (Brizze & Eik-Nes 1961) and pituitary stalk (Porter 1968); TSHRH (TRH) in SME and the whole hypothalamus (Averill & Kenredy 1967); FSHRH (FRH) in the pituitary stalk, SME and arcuate nucleus (Watanabe & McCann 1968); recently FSHRH has also been reported from the paraventricular neurons (Motta et al. 1971); LHRH (LRH) in SME, SON, supra-chiasmatic, paraventricular, arcuate, lateral hypothalamic area and mammillary bodies (McCann 1962) and has been prepared uncontaminated with other hypophysial hormones including FRH (Schally et al. 1966); in addition to the above it has also been located in the mesencephalon (Endcrozi & Hillard 1965); MSHRH in SME and paraventricular nucleus (Taleisnik et al. 1966); MSHIH in SON (Taleisnik & Tomatis 1967); and GHRH in SME and paraventricular nucleus (Schally et al. 1968).

Specificity of the Releasing Hormones

In vitro experiments have shown that there is a certain amount of specificity of action of these releasing hormones. For example, LH cells would react only to the

addition of LHRH and not to any other releasing hormone (McCann 1970). In passing, it may be noted that McCann et al. (1976) stated that specific prostaglandins at the hypothalamic (and hypophysial) level control the release of gonadotropin and prolactin.

The Releasing or Inhibitory Hormones or Factors

1. Corticotropin Releasing Hormone (CRH) (Saffran et al. 1955),
2. α_1 -Corticotropin Releasing Hormone (α_1 -CRH) (Guillemin et al. 1960),
3. α_2 -Corticotropin Releasing Hormone (α_2 -CRH) (Schally et al. 1962),
4. β -Corticotropin Releasing Hormone (β -CRH) (Schally et al. 1962),
5. Thyrotropin Releasing Hormone (TRH) (Shibusawa et al. 1959),
6. Follicle stimulating Hormone Releasing Hormone (FSHRH, FRH) (Igata-shi & McCann 1964),
7. Luteinizing Hormone Releasing Hormone (LHRH, LRH) (McCann et al. 1960),
8. Growth Hormone Releasing Hormone (GHRH) (Franz et al. 1962),
9. Melanocyte Stimulating Hormone Releasing Hormone (MSHRH) (Taleisnik & Orias 1965),
10. Prolactin Releasing Hormone (Pigeon) (PRH) (Kragt & Meites 1965),
11. Growth Hormone Inhibiting Hormone (GHIH) (Kulich et al. 1967),
12. Prolactin Inhibiting Hormone (PIH) (Pasteels 1961),
13. Melanocyte Stimulating Hormone Inhibiting Hormone (MSHIH) (Kastin 1965).

These releasing and inhibitory (?) substances are polypeptides as already said

and appear to be structurally different from the neurohypophysial octapeptides,—oxytocin and vasopressin in two features. Thioglycollate splits the disulphide bridge in a vasopressin and oxytocin molecule and inactivates it; this does not happen in CRH and LRH. Also no sulphur-containing amino acids have been found in any of the releasing hormones (McCann et al. 1965). Further, the releasing hormones are heat-stable and are slightly larger than oxytocin and vasopressin (Guillemin 1964). According to Guillemin (1972), less than 10^{-12} g quantity of these peptides is secreted. Further synthesis of TRF is not ribosomally controlled; the enzymes appear to 'put together' the tripeptide TRF.

Corticotropin releasing hormone (CRH): This is a small polypeptide containing all amino acids of vasopressin plus serine and histidine. There are no specific assays for this. The natural ACTH-releasing factor is a polypeptide similar to vasopressin (Guillemin 1957). In the dog, vasopressin does not appear to be a physiological ACTH releaser (Anderson & Egdahl 1964). Schally (1970) stated that in the posterior lobe, substances which are not vasopressin, release ACTH. He called them CRF. Substances in the hypothalamus also release ACTH and these are probably true CRH. Purification of CRH makes it lose its property while TRH, LRH and GRH do not. The hypothalamic CRH looks to be more an α -CRH than a β -CRH (Guillemin 1970). According to Motta et al. (1968), the brain extracts may have a corticotropin inhibitory hormone. On a weight basis, β -CRH is more active than α -CRH.

Thyroid Stimulating hormone releasing hormone (TRH) (Thyroliberin Mark 1976): This hormone has been synthesized and is a tripeptide:

Pyroglutamyl-Histidyl-Proline amide
Pyroglutamyl is also known as pyrroline

carboxyl; it can also be written as: Ovine TRH=PCA-His-Pro-NH₂ (Burgus & Guillemin 1970). The half-life of TRH appears to be 4 min. The chemical structure of this releasing hormone is given by Motta and Martini (1972) and is reproduced by me (figure 45). Reichlin and Mitnick (1973) propose a 'TRH synthetase' activity. This is enzymatic, forming peptide bonds governed by adenosine triphosphate. Experimentally it was possible to demon-

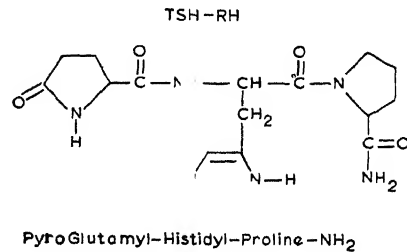


Figure 45 The structural formula of TSH-RH (After Motta & Martini 1972)

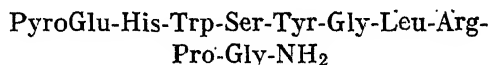
strate that the arcuate nucleus was the centre of TRH synthesis. Just as noradrenergic fibres of superior sympathetic ganglia control pineal enzyme function, TRH peptidergic neurons are controlled by monoaminergic nerve impulses. In sheep it has been noticed that synthetic TRH can release prolactin (Schally et al. 1973), also this has been noticed in rats and humans by other workers.

Recent researches (Oliver et al. 1974) have brought out that in the rat, the hypothalamus showed greatest concentration of TRH and other parts like PN, thalamus, brain stem, cerebrum, PD and cerebellum showed it in the descending order; the extra hypothalamic brain areas showed as much as 80%.

Follicle stimulating hormone releasing hormone (FRH): First described by Igaraishi and McCann (1964), this could be assayed by using the ovarian weight augmentation method (Steelman & Pohley,

1953). According to Motta et al. (1971) FRH and LRH are not synthesized in the ME; FRH is synthesized in the PVN and LRH in the suprachiasmatic and arcuate-ventrolateral areas. It has already been shown that both FRH and LRH are synthesized in the anterior hypothalamic area causing cyclical activity in the female mammal; FRH and LRH synthesized in the arcuate nucleus cause tonic secretion of gonadotropins in the male and female.

Luteinizing hormone releasing hormone (LRH) (Luliberin Marks 1976). This was first reported in the SME by McCann et al. (1960). This is a decapeptide. This could be assayed by using the ovarian ascorbic acid depletion (OAAD) method (Parlow 1961). Double antibody radioimmunoassay would be more precise, though laborious method of assay. The amino acid composition is as follows:



A molecule having LRH activity has also been synthesized. This hormone released both FSH and LH in rats (Schally et al. 1971); however, the amount of LH released was greater than FSH. It caused ovulation in golden hamsters. There was no evidence for a separate releasing hormone for FSH and LH. It has been designated FSH-RH/LH-RH. Both natural and synthetic LHRHs have major FSH activity *in vitro* (Reichlin & Mitnick 1973). Synthetic LH-RH/FSH-RH can stimulate synthesis of FSH-RH. There does not seem to be any species specificity of this hormone. It does not release GH, TSH, ACTH and does not exhibit prolactin release inhibiting activity. Peculiarly, Schally School reported that labelled LHRH was found concentrated in the pineal (Anand Kumar 1973).

The Milan School pointed out that as LH-RH is synthesized in the suprachiasmatic and arcuate ventro-medial region se-

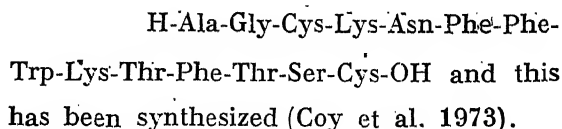
parately from FSH-RH (Motta et al. 1971), it is of opinion that there are two separate releasing factors, one for FSH and the other for LH. It has been pointed out by the Pittsburgh School (Dierschke et al. 1970) that LH is discharged in a pulsatile manner in ovariectomised monkeys. Greep (1973) also surmised that LRH was secreted in a similar manner. Malacara (see Barraclough et al. 1973) (during discussion) claimed an increase in LRH level prior to ovulation in women the LRF surge. The OAAD test for LRH referred to above is ineffective in birds and its use in testing crude extracts in the mammalian hypothalamus (McCann 1970) is questionable.

Growth hormone releasing hormone (GRH): Acid extracts of ME induced in sheep or beef increased release of GH into the medium in pituitaries incubated *in vitro*. Five minutes after injection of the hypothalamic extracts *in vitro* into rats, the GHRH caused not only release of GH but also caused synthesis of the hormone (Coates et al. 1970). It is a decapeptide and the synthetic decapeptide stimulated the release of GH *in vitro* measured by the 'tibia' test. The amino acid composition of GH is as follows:

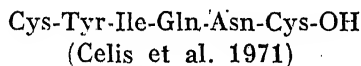


It has been synthesized also. This decapeptide corresponds to the amino terminal sequence of the porcine haemoglobin β chain. This has to be tested for GH activity.

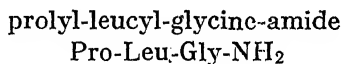
An inhibiting hormone of GH (Somatotropin) is also known. The somatotropin release inhibiting factor (SRIF) or Somatostatin is a cyclic tetra-decapeptide:



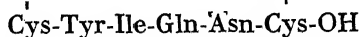
Melanocyte stimulating hormone releasing hormone (MSHRH): In the mammals, there are two melanocyte stimulating hormones, viz., α -MSH and β -MSH. Birds and mammals behave very differently with regard to MSH from other vertebrates. In the fish, Amphibia and reptiles, MSH affects the melanophores. The intermediate lobe cells are innervated by catechol aminergic and 'peptidergic' fibres; in the former the granules are smaller than in the latter. The intermediate lobe secretion is under the inhibitory control of the hypothalamus. In the mammals, the intermediate lobe (which may be absent in many including man), the MSH functions in a different way; there is no skin colour change. Of the two MSHs, α -MSH is a tridecapeptide and is not located in circulation while β -MSH circulates in the blood. The MSH in this group functions as an inhibitory one. The amino acid composition of MRH is as follows:



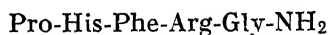
Melanocyte-stimulating hormone inhibiting hormone (MIH) (Kastin et al. 1973) (Melanostatin Marks 1976): Synthetic MIH has been prepared; it is a tripeptide and has an amino acid composition as follows:



In 1971, Bower et al. (1971) described the composition of MIH as follows:



In 1972, Nair et al. (1972) proposed a composition as follows:



MIH is rapidly inactivated. It can reduce MSH content in rats kept under constant illumination; exposure of rats to light increases pituitary gland MSH content,

probably mediated by the pineal. MIH injection causes decrease in plasma levels of MSH but an augmentation of pituitary MSH. Labelled MIH accumulates in the pineal, adrenal, kidney, liver and PI of the PG. Tocinoic acid (the ring structure of oxytocin) which inhibited MSF secretion from the PI of rats was tested but found lacking in 'melanocyte lightening' effect (MIH lightens dermal melanocytes in the frog's skin which has been previously darkened by removing the hypothalamus; on the other hand, removal of PI of the frog completely blanches the animal).

Prolactin release inhibiting hormone (PIH): After its discovery (Pasteels 1961), a specific prolactin release inhibiting hormone was described by Talwalker et al. (1963); they pointed out that *in vitro*, vasopressin, epinephrine, norepinephrine, acetylcholine, serotonin, histamine and brady-kinin could not mimic the action of PIH. The hypothalamus exercises a tonic inhibitory effect on prolactin secretion; the chemical nature of this inhibitory factor is not known. If the pituitary tissue is cultured, both prolactin and growth hormone are secreted. Extract of ME inhibits the release of prolactin and lactation ensues (McCann & Friedman 1960). In the anterior hypothalamus the suprachiasmatic region is one of the areas involved in the control of prolactin secretion (Pasteel 1970). The anterior hypothalamic area controls gonadal function and prolactin secretion and this area is both oestrogen and progesterone sensitive; thus there appears to be a link between the regulation of LH (FSH ?) and prolactin. Nicoll et al. (1970) pointed out that in the lizard that they studied, the neural control was one of hypothalamic stimulation for prolactin; it resembles the bird in this. Meites was not convinced of this argument.

This anterior hypothalamic area should not be confused with the anterior hypothalamic nucleus which according to Neumann et al. (1970) (see their figure 12, p. 590) is not oestradiol responding. Probably the neurons controlling gonadotropin release and prolactin inhibition in the anterior hypothalamic area function separately. In the human, growth hormone and prolactin are separate entities and the hypothalamus has also an inhibitory effect on prolactin (Neumann et al. 1970).

Releaser of Releasing Hormone (RH-RH): Some polyamines (spermine, putrescine) may cause indirectly, *in vivo* release of LH and FSH by causing release of FRH and LRH. These amines may be called releaser of releaser hormones (RH-RH or RH²) (Bogdanove 1970).

Tocinoic Acid

The ring of oxytocin, tocinoic acid, (H-Cys-Tyr-Ile-Glu-Asn-Cys-OH) inhibited MSH release from the PI of rats (Hruby et al. 1972). Kastin (1973) (during discussion, p. 28) pointed out that tocinoic acid (Hruby et al. 1972) was lacking MIH activity in the lesioned frog.

Other Peculiarities of Neurosecretion

LH, FSH, TSH and ACTH also show a certain amount of releasing potency (Szentagothai et al. 1968); further, in birds the hypothalamus may be manufacturing ACTH and storing it in the ME. Other hypophysial hormones are also seen in this region. The exact significance is not known.

The Polypeptide Hormones

The possible secretion of peptide hormones so far as is known (Sheela & Pandalai 1968) from the nucleus/nuclei is

as follows:

| | PON | |
|-----------------------------|---------------|----------|
| Fish and Amphibia | ADH, oxytocin | |
| | SON | PVN |
| Reptiles (<i>Calotes</i>) | -- | ADH |
| Birds | ADH | ? |
| Mammals | ADH | oxytocin |

In the mammals, oxytocin and vasopressin are present in both SON and PVN and the former hormone is more in camel, dog and sheep (Martini et al. 1959), it has already been brought out that the presence of little oxytocin in SON and a little vasopressin in PVN may be due to the incomplete separation of nuclei during extraction in some species of mammals (Heller 1963). In the toad, Rodriguez (1970) described the peculiar occurrence of ADH in the preoptic nucleus, neural lobe, CSF and choroid plexus.

The Input Neuron Pathways of SON and PVN Cells

A number of afferent fibres come from the brain and make synaptic contacts; these are mainly with the perikaryon, and axodendritic and axo-axonic contacts are also seen. The nerve endings show clear synaptic vesicles (500 Å) which contain acetylcholine and a few dense granules also. The cholinergic synapses on the soma are confirmed by the presence of cholinesterase in the SON and PVN. Norepinephrine fibres are also noticed in SON and PVN area but the magnocellular cells do not show amines. But Hökfelt (1968) feels that the nerve terminals containing dense core granules may be adrenergic ones.

Storage and Release of Polypeptide Hormones

Oxytocin and vasopressin are stored in the axonal ends in the PN. What is the

stimulus for the release of hormones, individually or together is not clear. Suckling the dog and rabbit appears to release both the hormones. Long lactation in the rat appears to exhaust oxytocin but does not affect ADH. A structural formula of oxytocin (figure 46) is given as it helps in the study of the evolution of posterior lobe hormones.

Evolution of the Octapeptide Hormones

The evolution of the neural lobe hormones which are octapeptides may not be out of place here. We have to take into account at least 7 hormones which appear to have originated from a parent molecule. A single gene duplication and substitution in positions 3, 4 or 8 lead to two molecular 'lines'. The parent molecule is as follows (Frieden & Lipner 1971):

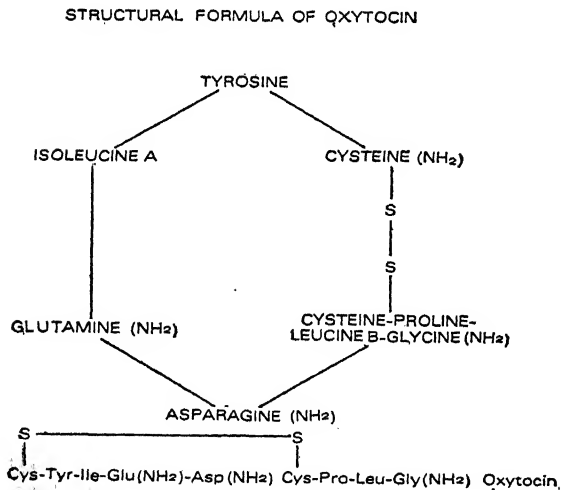
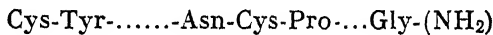


Figure 46 The structural formula of oxytocin (After Gorbman & Bern 1962)

Peyrot (1968) shows the interrelationship of the hormones in the form of a

family tree (figure 47). According to Heller (1964), peccary (American wild pig) exhibits both lysine and arginine vasopressin and according to Stewart (1968) the Peru strain of mouse exhibits lysine vasopressin. In 1974, Heller gave an account that adult mammals show arginine vasopressin; foetal neurohypophysis shows vasotocin (sheep, seal, guinea pig pig). Pavel (1971) showed that the pinea stalk produces arginine vasotocin in bovines and this is secreted into the CSF by the ependyma. Sawyer (1966) put forth a scheme of classification among the artiodactyls (figure 48). The latest account

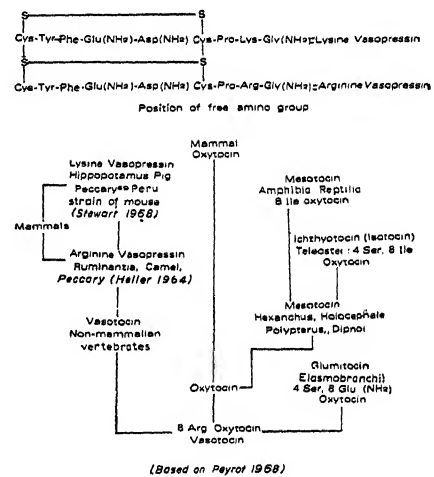


Figure 47 The interrelationship of the posterior lobe hormones (After Peyrot 1968)

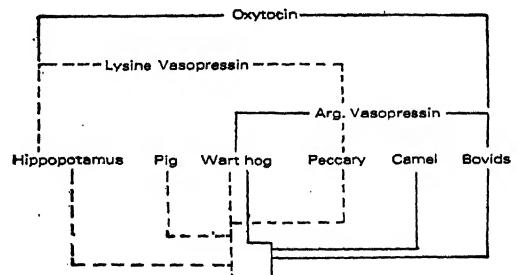
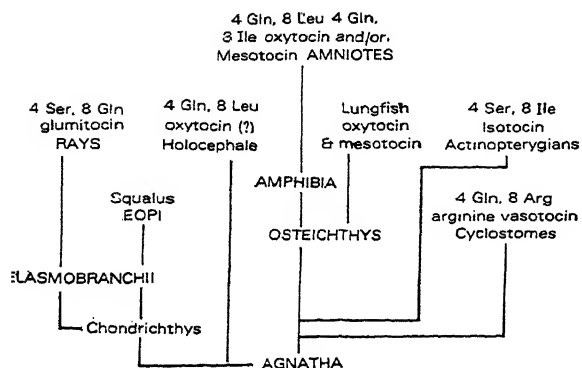


Figure 48 The posterior lobe hormones of ruminantia (After Sawyer 1966)

is given by W. H. Sawyer as reproduced by Perks (1969) on p. 190. This brings out that the cyclostomes have only the basic arginine vasotocin and the sharks and rays appear to have different neurohypophysial peptides (figure 49).

The amino acid composition of the octapeptides according to Heller (1974) is shown in figure 50.



The distribution of neutral neurohypophysial principles mainly in fish; the rays and sharks appear to have different neurohypophysial principles.

Figure 49 The evolution of the posterior lobe hormones (After W. H. Sawyer, see Perks 1969)

Neurohypophysial Hormones

| | Aminoacids in position | | |
|----------------------------------|------------------------|-----|-----|
| Basic peptides | 3 | 4 | 8 |
| Arginine vasopressin | Phe | Glu | Arg |
| Lysine vasopressin | Phe | Glu | Lys |
| Arginine vasotocin | Ile | Glu | Arg |
| Neural (=oxytocin-like peptides) | | | |
| Oxytocin | Ile | Glu | Leu |
| Mesotocin | Ile | Glu | Ile |
| Isotocin (Ichthyotocin) | Ile | Ser | Ile |
| Glutitocin | Ile | Ser | Glu |
| Valitocin | Ile | Glu | Val |
| Aspartocin | Ile | Asn | Leu |

(After Heller 1974)

Figure 50 Aminoacid compositions in positions 3, 4 and 8 of the posterior lobe hormones (After Heller 1974)

The Mode of Formation of Neurosecretory Material

The 'raw' protein material is synthesized by the endoplasmic reticulum and is passed on to the Golgi apparatus. The latter organizes the protein NSM into a supra-molecule; it becomes membrane limited, the electron dense granule representing the packaged NSM. The Golgi membrane in the perikaryon may also fragment and form vesicles; these are small and electron lucent to start with and later they may synthesize NSM which is electron dense. For synthesis, vasopressin is dependent on the perikaryon and passes through significant biosynthetic changes during its passage into the axon (Bern & Knowles 1966). Sloper (1966) (p. 160) also started that neurosecretion appears widely distributed in the perikarya. Bern et al. (1962) also say that the granules 1000 Å are found in the region of Golgi apparatus and are occasionally included in membranes, tubes or cisternae of which the apparatus is composed. Thus synthesized in the perikarya, the NSM passes into the axon where the axoplasm exhibits a proximo-distal flow of the material and terminates near blood capillaries in the ME or PN or at glandular cells in the PI (neurosecretomotor innervation). This is called the 'transport hypothesis'. There is also a view that the axon may synthesize NSM, the granule reaching a maturation size (Sachs 1959). The tubular-lamellar system in the axon may synthesize NSM and/or form local axonal neurosecretory granule (De Robertis 1962). Electron microscope findings modified the above axonal synthesis view to 'a packaging and pulling together of amino acids and/or polypeptides and proteins with the neurotubules considered to be continuous with the Golgi apparatus' taking place in the distal part of the neurons (Dellman &

Owsley 1969); the same authors also state that though the neurotubules may be possible sites of packaging, it is likely that they do not contribute extensively to augmented neurosecretion.

In the elasmobranchs and teleosts, the neuronal nucleus may also contribute NSM (see figure 2, p. 24)—(Bern et al. 1962). The nucleus appears to participate in the formation of NSM; fragments of it are thrown into the cytoplasm (Kobayashi et al. 1968).

The Neurosecretory Material

The NSM is a protein. This macromolecular chemical may be a glycoprotein, lipoprotein or a glycolipoprotein. This functions as a carrier of a peptide of a lower molecular weight which is the actual hormone. Neurophysin first isolated by van Dyke et al. (1941) and later purified by Acher et al. (1956) is the inert carrier of the neurohypophysial octapeptide hormones. This inert carrier may also act as the precursor or parent substance for the neurohypophysial hormones (Bern & Knowles 1966). It may be mentioned here that the neural lobe hormones are cyclic cystine containing octapeptides while the RHs and IHs are polypeptides.

Extrusion of NSM from the Axonal Bulb

The terminal bulb of a neurosecretory axon may show, in addition to membrane bound electron dense NSF, certain vesicles. De Robertis (1964), Koelle (1961), Oota (1963) and Kobayashi et al. (1970) consider them to be synaptic vesicles; the acetylcholine in them probably acts on the axon membrane and neurosecretory granule envelope during the process of extrusion. Holmes and Knowles (1960), Bern (1963) and Lederis

(1964) consider these as products of granule and vesicle fragmentation. Both synaptic vesicles and fragmentation products may occur together in an axon. There is also a view that microvesicles (synaptic vesicles) arise from microtubules (Vollrath 1970). In the sciatic nerve of the frog, synaptic vesicles arise in the Golgi apparatus and move to axon terminals by axoplasmic flow (Bræmner et al. 1958).

In response to nerve impulses, the synaptic vesicles present in axon terminals change their shape and number and liberate acetylcholine; the latter probably affects the permeability of the granule membrane to the neurohormone. Acetylcholine has a vasomotor function related to hormone release. Electron microscope discloses that the outer coat becomes one with the plasma membrane and the granule gets into the perivascular space (Hartmann 1958). Axonic terminals in rats and toads (Palay 1957, Gerschenfeld et al. 1960) contained electron dense NSM and certain electron lucent neurosecretory granules. The release of granules is by molecular dispersion and the discharge of the whole granule has never been observed. Though this 'intracellular' hypothesis (an intracellular breakdown of hormones of the neurosecretory granule and its subsequent passage through the cytoplasm and plasma membrane) is difficult to accept, the granule extrusion is gaining ground (Douglas 1967). The neurosecretory granule bound with the peptide neurophysin escapes into the perivascular space (where the two are separated). The same exocytotic method of extrusion is also seen in the chromaffin cells of the adrenal medulla (Douglas et al. 1971), which would go to support what happens in the PN.

The axon terminals (figure 51) near capillaries and basement membrane show a pitted appearance, very suggestive of

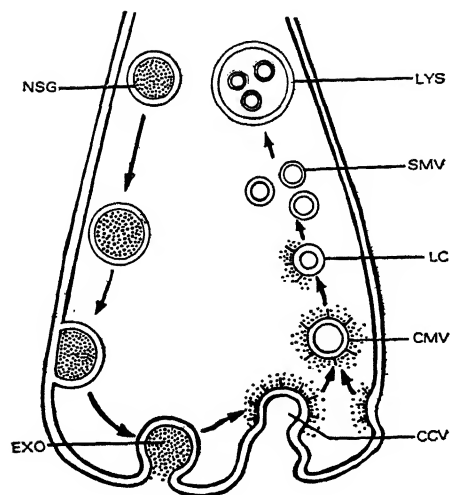


Figure 51 The extrusion of the neurosecretory material (After Douglas et al. 1974, modified)

exocytotic activity in other cells (Nagasaki et al. 1970). The granule covering, where the granule is to be extruded, fuses with the plasma membrane and opens out by a narrow space which later assumes an omega shape, while the electron dense material or granule is becoming less and less electron dense. Inward budding at such pits is also noticed giving rise to microvesicles by pinocytotic activity, (endocytosis). Douglas (1974) has restated the above in a slightly altered way. The neurosecretory granules with their outer membrane come near the plasma membrane in the neurohypophysis and the two membranes fuse. Fusion is immediately followed by fission and the granule is extruded by exocytosis. The remaining granule membrane becomes incorporated in the plasma membrane and does not seem to increase its surface area. The granule membrane now becomes coated (caveolae) and these microvesicles are pinched off by micropinocytosis or Douglas prefers the term vesiculation for this. These microvesicles lose

their outer coating and become smooth synaptic vesicles. These synaptic vesicles are digested by the lysosomes. This observation is opposed to the 'cytoplasmic pool' hypothesis which assumes a depolarization and influx of Ca^{2+} , then a repolarization followed by intracellular re-equilibration.

It is very interesting to note that undischarged secretory granules in neurons and in PG cells get fused with lysosomes and the phenomenon is called crinophagy. Temporary excess of secretory granules is therefore taken care of by lysosomes (Farquhar 1971).

Akert (1971) stated that the exact mode of contact between plasmalemma and the vesicle is not known; it is likely that there is a tight junction between the two and a 'synaptopore' may represent the diffusion channel rather than a 'synaptic' exocytosis.

It may not be out of place here to mention how neurotransmitters become external from the varicosities in the case of catecholamine nerve fibres. Very much like acetylcholine is spurted into the synapse when the nerve membrane is depolarised due to nerve stimulation, the release of noradrenaline (NA) follows the same path. The vesicles at the terminals of sympathetic nerves contain not only NA but also enzyme dopamine-beta-hydroxylase (DBH) which converts Dopa into NA. When the nerve is electrically stimulated, NA is released. Certain agencies also prevent the release of neurotransmitters and enzymes and the absence of calcium is one such agency. It is likely that depolarization causes calcium to activate a contractile filament on the neural membrane (as it does the microfilaments of muscle cells) which contracts to make an opening through which the soluble contents of the vesicle are released (Axelrod 1974).

Action of the Releasing Hormone at the Pituitary Cell Level (McCann et al. 1968)

The various RHs reach the PD through the portal circulation and come in contact with the membranes of the pituitary secreting cells. This causes the elevation of K^+ and the depolarised membrane permits the uptake of Ca^{++} and this triggers the release of stored granules. Sodium, in place of potassium, cannot do this. The discharge of secretory granules into the blood somehow causes the biosynthesis of fresh hormone inside the cell. It is not clear how the inhibiting hormone works; probably the membrane is hyperpolarised.

Neurophysin and Decapeptide Hormones

It is now common knowledge that both oxytocin and vasopressin are synthesized in the hypothalamic nuclei; more of vasopressin in SON and more of oxytocin in PVN the synthesis of the latter occurring throughout the length of the neuron (Lederis 1962).

In the SON, a protein inactive precursor to vasopressin is synthesized on the polysome (ribosomal RNA) of the endoplasmic reticulum; this is carried to the Golgi and there incorporated into secretory granules. Vasopressin probably is released inside the granules by the precursor (or parent substance (Bern & Knowles 1966)) during transport of granules to the periphery. This precursor substance is different from the carrier substance (Follenius 1965).

The two octapeptides bound together by neurophysin, the carrier protein (which also appears to be synthesized in the perikaryon of SON and PVN) move to the posterior lobe through the neuraxons. This neurophysin, which is a cystine rich protein, was first isolated from the poste-

rior lobe by van Dyke et al. (1941) as already stated; it was shown subsequently that this protein binds only vasopressin and oxytocin.

Direct release of the bound hormone which is physiologically inactive may take place; an indirect release by the cytoplasm would dissociate the hormone from the binding protein when the former would be active (Chard 1971).

Histochemical dyes stain the carrier protein; histologically demonstrable NSM is none other than neurophysin.

Stimulation of Neurosecretory Neurons

Recent researches on the caudal neurosecretory neurons of fish, the hypothalamic neurons of *Carassius* and of mammals have shown that at least some neurosecretory neurons can conduct impulses and this may determine neurohormone release. Both intracellular and extracellular determinations of neurosecretory cells and their processes indicate the long duration of action potentials, two to ten times more than the adjacent neurons. Hypothalamic nuclei of mammals have not shown longer than normal action potentials (Bern & Knowles 1966).

The Biogenicamines and their Neurons

These amines appear to modulate the secretion of hypophysiotropins. The *in vitro* and *in vivo* experiments on rats (both sexes) using amines showed similar results (Schneider & McCann 1969, 1970). It may be necessary to describe the amines in detail as there are two conflicting schools of thought.

The Neurons: Three different types of neurons containing dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) are known (Fuxe 1965); the Fuxe group (Stockholm School) have also des-

cribed other neurons (epinephrine, histamine) in the hypothalamus. DA cell bodies are found in arcuate and anterior periventricular hypothalamic nuclei. Innervation from the tubero-infundibular system is found in the external layer of the stalk. NE cell bodies are found in the pons and medulla oblongata. Recently Fuxe et al. (1976a) have shown NE nerve terminals in the subependymal, median and lateral external layers of the ME (figure 52). They also suspect that the median DA pathway may act as terminal for PIH. 5-HT cell bodies are found in the nucleus raphae dorsalis and nucleus raphae medianus. The axons ascend in the midbrain and innervate the hypothalamic nuclei. Dense terminals are seen in the nucleus supra-chiasmaticus. There is a high concentration of 5-HT in the arcuate nucleus. These are probably involved in regulating oestrous behaviour and controlling the peak secretion of LRH necessary for ovulation (Wurtman 1970). Serotonin injected intraventricularly (Schneider 1969, 1970) causes lowering of gonadotropins as stated above (inhibiting the RH) and stimulates prolactin release by inhibiting the IH.

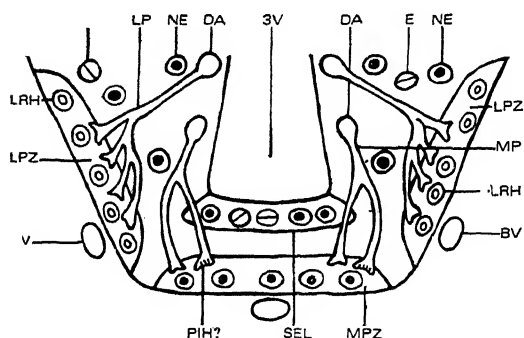


Figure 52 Schematic diagram of the distribution of Dopamine, Norepinephrine, Epinephrine and LRH in the lateral and medial parts of the rat median eminence, (After Fuxe et al. 1976, modified) Dopamine terminals are probably one of the prolactin inhibiting hormones. Acetylcholine is not shown

Stimulatory influence is caused through catecholaminergic pathway during ovulation (Zolovick & Labshetwar 1973). Pineal appears to control the release of prolactin; melatonin coming from the pineal increases prolactin level during the night as the pineal is active only during the night. Melatonin in large doses blocks ovulation (McCann et al. 1973).

The McCann School (1969) from their incubation experiments described that DA increased the release of LRH; NE and 5-HT failed to increase LRH and epinephrine (E) reduced the level of LRH. Injections of amines into the 3rd ventricle caused the following effects under experimental conditions (McCann et al. 1972). DA elevated both LRH and LH levels; in the males it elevated FRH and FSH levels; in lactating females, it lowered plasma prolactin. NE was much less effective than DA; E was least effective in elevating levels of FSH and LH and depressing prolactin. In the pro-oestrous rats, with ovulatory hormones blocked by pentobarbitol, EN injected into the 3rd ventricle caused ovulation; DA did not. 5-HT inhibited LH release (Schneider & McCann 1969). It was also brought out by Porter et al. (1972) that within ten minutes of injections of DA, LH increased fourfold; within 20 min., it increased 9-fold; within the next 30–60 min., there was a fall and again at 120 min., there was a 20-fold increase. Similarly at 90 min. FSH increased by 10-fold and then there was a fall. Under similar circumstances, prolactin was reduced in its release. Working on the rodents, the Sawyer School (Rubenstein & Sawyer 1970) reported that DA actually blocked ovulation in some. In 1972, the McCann School (Kalra et al. 1972) changed its view about DA and came to the conclusion that in the rat NE was involved in the release of LRH. It is interesting to note that Miyachi et al. (1973) repeated

the experiments of Schneider and McCann (1969, 1970) and could not confirm their results. The Stockholm School (Fuxe & Hökfelt 1970) is also opposed to the view that DA enhances LH secretion put forward by the McCann School. According to the former School, the axons of the tubero-infundibular nuclei are short and form complexes in the external layer of the ME close to the primary plexus. The axon terminals of these DA neurons make axo-axonic contact with axon terminals storing peptidergic releasing inhibitory factors controlling gonadotropin secretion; the latter belong to neurons which synthesize neurotransmitter substances. According to the Swedish workers, DA does not act directly upon the anterior pituitary and it *inhibits* the secretion of LRH and/or FRH. These DA neurons are activated by oestrogen, testosterone (castrated animal) and prolactin. According to Glowinski (1970), the DA neurons appear to activate the release of hypophysiotropic hormones into the portal circulation and NE appears to precede the DA activity.

The McCann School (1976) maintained at the Simla Conference, 1974, their previous stand that DA could serve as a transmitter to release LRH by axo-axonal transmission since dopaminergic neurons are present in the arcuate nucleus and that the tubero-infundibular tract ends in the external layer of the ME. At the same Conference, the Stockholm School (Fuxe et al. 1966) maintained that dopaminergic nerve terminals in the lateral layer of the ME *inhibited* LRH. They also pointed out that the dopaminergic fibres in other parts may control prolactin secretion. At the Brussels Conference, Fuxe et al. (1977) brought out that the hypothalamic noradrenergic mechanism is facilitatory and that of dopaminergic is inhibitory in the ME and further, that the nucleus accumbens may cause the inhibition of prolac-

tin secretion.

Recently Vijayan and McCann (1977, in press) have again brought out that the three catecholamines DA, NE and E can elevate LH and that the latter two are more effective. Of these, NE appears to stimulate the preovulatory release of LH. They feel that the evidence that DA inhibits LRH release as put out by Fuxe et al. (1977) at the same Conference may be due to measurements of DA turnover by histochemical fluorescence techniques in animals whose DA synthesis was blocked.

Motta et al. (1973) have brought out in their *in vitro* studies on rats that cholinergic mediators operate in the release of gonadotropin releasing factors. They found that the anterior pituitary plus two media eminentia (plus basal hypothalamus) and plus acetylcholine brought about a significant release of FSH; when cholinergic blocker like atropine was used in place of acetylcholine, FSH release was considerably diminished.

The Ependyma Cell

Recent researches (Knowles & Anand Kumar 1969) have brought out that specialised ependyma cells lining the 3rd ventricle in the rhesus monkey have a neuroendocrine function. When oestrogen level is high due to gonadal activity, certain tanycyte (branching) ependyma cells show a dilatation of plasma membrane indicating probably absorption and of increased activity. It has been shown that tritiated oestradiol given intramuscularly was detected in the CSF. These special tanycyte ependyma cells act as receptors to oestrogen and play a role in pituitary feedback control. In the latter, it is likely that these tanycyte ependyma cells absorb from the CSF and secrete into the portal vessels of the ME by their long processes by making synaptoid contacts;

the ependymal processes may also make a synaptoid contact with the PT cells (Anand Kumar & Thomas 1968). This is a new pathway for the NSM to affect the PG. This transependymal transport from the 3rd ventricle to the portal vasculature has been further confirmed by Kobayashi et al. (1972) who described the uptake and transport of horse-radish peroxidase by the ependyma cells; the presence of neurophysin and gonadotropin releasing hormone in the dynamic tanycyte ependyma cells of the mouse ME (Zimmerman et al. 1974) has also brought out the intimate relation between the CSF and the portal circulation.

These specialised tanycyte ependyma cells differ in the two sexes and change during the menstrual cycle. These regress when the female is ovariectomised and regain normalcy with a single injection of oestradiol. The bleb-like processes from the ependyma cells projecting into the 3rd ventricle increase at the time of ovulation and also shortly after ovulation (Anand Kumar 1968, Anand Kumar et al. 1973).

During discussion on the secretory cells in the ependyma (Knowles 1971), Dellmann brought out that the ependymal and subependymal cells in a transected frog neurohypophysis may differentiate into nerve cells which are Gomori negative. Vigh-Teichman and Vigh (1970) reported that the ependyma adjacent to the arcuate nucleus is very different. This may be the region where the neurons are throwing substances into the CSF,—forming the "liquor contacting neuronal system".

In the rat injection of LRH into the 3rd ventricle released LH; this experiment in addition to proving the above also brought out that direct uptake of LRH from the 3rd ventricle into the hypophysial portal system is not physiologically a significant process (Weiner et al. 1972).

Rodriguez (1972) referred to two modes of ependymo-vascular transport. The basal processes of the ependyma may reach the blood vessels or the processes from the perivascular basement membrane may reach the ependyma. Transport from the CSF may pass through the processes of the ependyma into the portal blood vessels. Transportation in the opposite direction is also not ruled out according to him.

According to Clementi and Ceccarelli (1970) the fine structure of the tanycyte ependyma cell shows that it is not secretory and has probably other physiological functions.

The Pituicyte

Bucy (1930) was the first to describe the independent nature of the glial (neuroglial) cells in the neurohypophysis and called them pituicyte. Some Herring bodies are merely neurosecretion filled pituicytes. In the neural lobes of frogs, reptiles, birds and mammals pituicytes are commonly noticed (Wingstrand 1966).

In the above four classes of vertebrates, the PN is independently vascularised. The hypothalamo-hypophysial neurosecretory fibres terminate near capillaries in the neural organ. However, it is noticed that the terminals of fibres deeply imbed themselves in the glial cell which thus interposes itself between the terminals and the blood vessel. What exactly is the function of the glial cell, it is not clear (see Gorbman & Bern 1963, p. 359 for figure).

Neural Structures or Pathways Modulating the Activity of the Hypophysiotropic Hormone-Producing Neurons

The release of gonadotropic hormones (FSH, LH) has been studied in great de-

tail in the rat (Mess et al. 1970). In the male, there is only a tonic secretion of FSH and LH. This maintains the weight and histology of the testis in normal state. The small neurosecretory cells of the hypophysiotropic area synthesize FRH and LRH and a tubero-infundibular tract carries these hypophysiotropins to the portal vessels. Also in the arcuate nucleus which forms a part of the hypophysiotropic area, dopamine neurons are present; the amine is sent into the portal vessels by a separate tubero-infundibular tract. In the female rat (Flerkó 1970), the reproductive activity is cyclical as pointed out previously and the oestrous cycle repeats every 4 or 5 days. There is an oestrogen and gonadotropins surge prior to ovulation. The latter phenomenon is brought about by certain FRH and LRH neurons in the anterior region of the hypophysiotropic area. The FRH neurons are triggered by afferents coming from cells in the anterior hypothalamic area; the LRH neurons are triggered by afferents coming from the cells in the preoptic area. Norepinephrine appears to be the triggering agent coming from the anterior hypothalamic and preoptic areas. This acts upon the neurons in the hypophysiotropic area which release FRH and LRH to cause a gonadotropin surge. Therefore, NE appears to act as the synaptic transmitter and not DA. The oestrogen surge appears to enhance greater NE synthesis in the neurons of the preoptic and probably also the anterior hypothalamic nuclei.

The axons from the FRH and LRH neurons are a part of the tubero-infundibular tract which enters the ME and ends up near the portal vessels and causes the so called surge secretion of FSH and LH which in turn causes ovulation. That these relations as described above was proved by an ingenious experiment by the

Hungarian School (Halász et al. 1962). A bayonet-shaped knife usually called Halász knife mounted on a Horsley-Clarke stereotaxic apparatus was used. The knife cut round the hypophysiotropic area including the stalk ME and isolated it or 'deafferented' it. Though thus isolated from the brain, the hypophysiotropic island maintained the basal function of the hormonally controlled target organs except the ovary. Ovulation and some other rhythmic endocrine functions were impaired; vaginal cytology showed permanent oestrous. Absence of ovulation was explained by the fact that enhanced or surge release of FSH and LH necessary for it was not there as the NE triggering mechanism of the FRH and LRH neurons was disconnected by the knife. Martini (See Discussion on Reed's paper, *Mem. Soc. Endocrin.* 19. 892, 1971) brought out that there were two levels of control of LH-RH secretion. One was hypothalamic and the other was extrahypothalamic.

The Male and Female Hypothalamus

The hypothalamus may be categorised as male and female. In a large number of animals including women (the female rabbit, cat, ferret, mink, ground squirrel being reflex or induced ovulators are non-cyclic) in the female it is cyclic or there is a periodic surge or peak discharge of gonadotropins leading to ovulation (Harris 1964). The hypothalamus also monitors a cyclic activity of the vagina caused by the cyclic secretory activity of the ovary and the cyclic period of sexual receptivity (Gorski 1970). There are two centres (table 1) in the female hypothalamus, one controlling tonic (the arcuate nucleus mainly) and the other, the cyclic activity (the anterior hypothalamic and preoptic areas) of the PG and in the male, one centre situated in

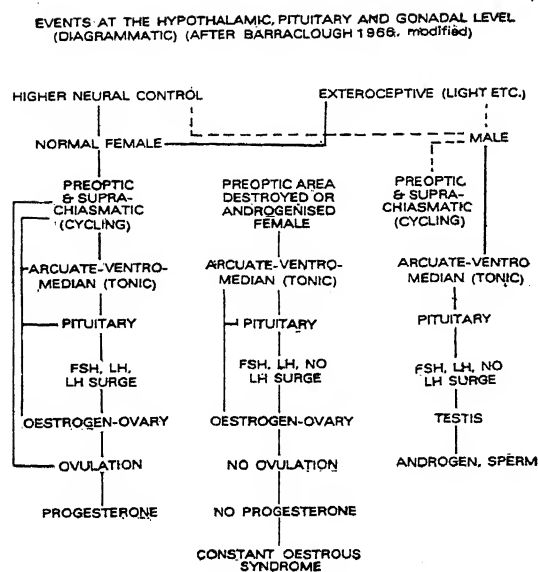


Figure 53 The cyclic and tonic release of gonadotropins in a male and female mammal. Broken lines indicate nonfunctional areas (After Barraclough 1966, modified)

the hypophysiotropic area (the arcuate nucleus mainly) (Barraclough 1966). If the male PG is transplanted into the hypophysectomised female sella turcica, the female hypothalamus induces cyclical behaviour in the transplant. The differentiation into the male hypothalamus is brought about by the testicular androgen at a critical period of the differentiation of the CNS (Harris 1964).

The cyclical discharge of gonadotropins is not that simple as it may appear at the surface. In addition to the preoptic area functioning as the agency responsible for it, the limbic system also plays its part. It may be said that there are broadly two components, one facilitatory and the other inhibitory in this. The former coincides with oestrogen neuron system where fluctuating electrical activity is also noticed during the oestrous cycle. Further, in the facilitatory one, there are

two facets: one consisting of the medial amygdala, the accumbens and bed nucleus of stria terminalis and the arcuate nucleus are oestrogen sensitive, while medial preoptic area and the septal nucleus are oestrogen refractory. However that may be, both these areas could induce gonadotropin release under suitable conditions, e.g., electrical stimulation. In contrast to this, the hippocampus, the amygdala and central gray matter,—all parts of the limbic forebrain structures act as inhibitory system. Cyclicity could be viewed as a representation of push-pull interaction between the above two systems (Kawakami et al. 1976). In the rat brain (Pfaff 1976), the oestrogen sensitive cells are shown by using labelled oestrogen. In Pfaff's (1976) figure 1, the lateral septum does not show any oestrogen receptive cells while the median preoptic area is loaded with them differing thereby from Kawakami et al. (1976). This has to be decided by future work.

The primates including man behave in a different way. It is a common knowledge that in the female primate, there is a pre-ovulatory LH surge as in other mammals. If, however, the males are castrated in adulthood and oestrogen administered, there is first a drop in the elevated plasma concentration, there is a rebound of the hormone to control levels and then a decline unlike the similarly treated females (Karsch et al. 1973). In immature monkeys such an LH surge cannot be induced by oestrogen. However, the adult male primate may be made to cycle its gonadotropin secretion (Karsch et al. 1973), if the influence of the testicular androgen is removed and a larger dose of oestrogen than in the above experiments be given. This result cannot be achieved in the rat castrated in adulthood. This leads one to conclude that the trigger mechanism referred to above is switched off by the

neonatal androgen action in the male mammal.

Bogdanove (1972) feels that in castrated monkeys where there is no positive ovarian pituitary feedback signal, LH surges cannot be induced, it is difficult to assume that an oestrogen surge is necessary for an LH surge. According to him, the oestrogen peak is merely an effect and not the cause of LH surge. It has now been proven by the Knobil School (1974) that oestrogen is the cause of FSH and LH surges; for this plasma oestrogen concentration has to be sustained for at least 12 hr. and has to exceed approximately 60 pg/ml (Yamaji et al. 1971). The Knobil School (1974) has also examined if in the rhesus monkey the positive feedback mechanism of oestrogen leading to gonadotropin surges and the negative one leading to tonic secretion of the same are comparable to those in the rat where cyclic secretion is controlled by the preoptic area. By an ingenious modification of the Halász technique, they have been able to deafferent the median basal hypothalamus and come to the conclusion that in the monkey a preoptic area signal is not necessary for cyclic release and there is uninterrupted ovulatory cycles differing thereby from the rat. They have also pointed out that environment (daylight-darkness) does not seem to play a major role for the timing of the reproductive processes in the primates. Further, if oestrogen is injected into these deafferented monkeys, there is a surge of LH. They also retain the circroral pulsatile pattern of LH secretion and this episodic phenomenon appears to be an autonomous possession of the medial basal hypothalamus. On the other hand, Norman and Spies (1974) who did similar deafferentation experiments with monkeys could not confirm the LH surge either spontaneous or after oestrogen injection. With regard to

the pulsatile pattern of release of gonadotropins, Yen (1976) brought out that it was caused by pulsed secretion of LRH, this pattern is also seen in the portal blood of the monkey, it can be abolished with antiserum to LRH.

Recent research (Goodman et al. 1977) has brought out in the monkey that the mid-cycle surges of oestrogen and of gonadotropins are not required for subsequent initiation and synchronization of folliculogenesis. A ripe follicle or corpus luteum in the ovary appears to have an inhibiting influence on the growth of further follicles.

It has also been found (Dierschke et al. 1973) in the monkey that the negative feedback control of tonic secretion of gonadotropin by oestrogen had different pathways from those of the positive feedback control of cyclic gonadotropin secretion; this negative feedback loop involves very likely a dopaminergic and/or α -adrenergic component (Knobil et al. 1972).

The Hypothalamus and its Relation to other parts of the Brain

The hypothalamus and the other parts of the brain are connected by fibre connexions. We may divide these into afferent and efferent pathways (Raisman 1970). The afferent connexions are the fibre tracts ascending to the hypothalamus from the brain stem. Fluorescence technique discloses the catecholamines (epinephrine or adrenaline) and 5-HT (Serotonin) containing fibres (serotoninerigic) ascend by the above route into the forebrain. From the forebrain to the hypothalamus, there is a limbic pathway with the associated amygdala, hippocampus and pyriform cortex. Probably there are no fibres coming from the neocortical area into the medial hypothalamus. Arising from the amygdala (figure 54) is the stria terminalis

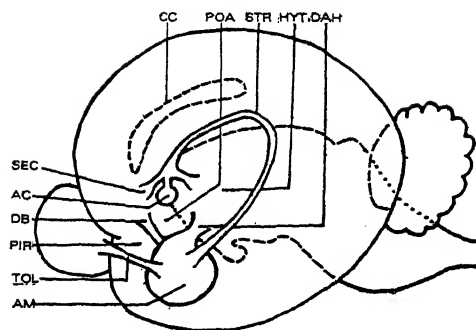


Figure 54 Amygdaloid complex and its connexions, diagrammatic (After de Groot, J, *J. Neuroendocrinology*, vol. 1, eds L. Martini and W. F. Ganong, Academic Press, New York, 1966)

pathway; the strial fibres arise from the amygdala and after sending a few fibres to the anterior commissure, enter the hypothalamus and end in the mammillary nuclei. From the region inferior of the hippocampus, the fibres after traversing the various parts come as far as the anterior hypothalamic level. A special area of the hippocampus gives rise to fibres of the medial corticohypothalamic tract. This fibre tract travels up to the dorsal aspect of the suprachiasmatic nucleus and then proceeds to end near the arcuate nucleus.

Two afferent pathways terminate in the medial hypothalamus—the stria terminalis in the ventromedial nucleus and the medial cortico-hypothalamic tract in the arcuate nucleus.

The Sensory Pathways

Odour plays an important role in reproduction in mammals. The olfactory bulb receives fibres from the olfactory nerve and projects to the olfactory tubercle, the pyriform cortex and also to the cortico-medial group of amygdaloid nuclei, from which there are two groups going to the hypothalamus: the ventral amygdaloid-

fugal pathway and the stria terminalis.

A direct visual projection to the hypothalamus in mammals is yet to be proven. [With regard to ambient light and light and dark periods, the pineal seems to play its part in this; it acts by a humoral pathway rather than a hypothalamic afferent fibre pathway. Where the visual input is more complex (e.g., behaviour), this information reaches the hypothalamus involving the neocortex. The hippocampal projections to the hypothalamus provide an indirect route for neocortical influence upon the hypothalamus. This goes to the entorhinal area where the major afferents to the hypothalamus arise.

The efferent connexions are (a) the posterior PG and (b) the median eminence of the neurohypophysis.

The Posterior Pituitary Gland

The best understood efferent system is the magnocellular hypothalamic supraoptic and paraventricular fibres running to the posterior PG. These fibres end in a palisade around the capillaries of the neural lobe. Large electron dense vesicles (2000 Å—3000 Å diameter) are present in the axon terminals here. Abundant adrenergic and dopaminergic afferent synapses on the SON and PVN perikarya and on the axons are seen. These afferent fibres probably come from the median forebrain bundle (Fuxe & Hökfelt 1967).

The Median Eminence

The parvicellular system containing the ventromedial nucleus, arcuate nucleus and the anterior periventricular nucleus from the hypophysiotropic area send axons which palisade around the primary plexus of the portal system in the external layer of the ME.

The ME and PN (both independently vascularised by arterial branches) are closely linked; extirpation of the PN results in the accumulation of NSM in the ME. A new PN may be formed and blood vessels grow into this area and endings of nerve fibres at the blood vessels are seen. The ME as already said, is an anatomical interface between the brain and the PG.

The Magnocellular Nuclei (SON, PVN)

In the vole (Clark & Kennedy 1967), the PVN is divided into two groups and the neurosecretory system is more active during the breeding season. The SON of the Indian squirrel (Vijayan & Sathyanesan 1971) is seen in three groups. The PVN axons could be traced to the SON in the squirrel and then to the median eminence. Beyond this their identities could not be made out. In the human, it has been noticed that the destruction of the SON caused the disappearance of PVN suggesting that many PV fibres end in SON (Baker & Craft 1940). The neurons of the SON in the human (Daniel 1966) are considerably large with nucleoli. The neuron number is between 50,000 and 70,000. If the pituitary stalk is cut, many neurons disappear. A nerve cell dies if its axon is cut but if it has a collateral branch above the level of transection, it does not disappear. If the transection is higher up, a severe loss of nerve cells is seen though never complete. The PVN may have 39,000—54,000 neurons. These show both large and small cells. The fibres of these pass through the SON and form the hypothalamo-hypophysial tract. If the stalk is transected, the large cells disappear. In the rhesus monkey, Palay (1953) described that the PVN contained large number of heavily granulated neurons than the SON. Neuraxons from the PVN and SON

containing NSM pass through the ME and the stem into the PN where they end near the blood capillaries. The hypothalamo-hypophysial tract could be traced from the two SO and PV nuclei into the PN. None of the fibres was noticed to end near the primary plexus in the stalk. No multinucleate neurons in the PVN were seen as occasionally such cells are met with in the human.

The Neurosecretory Material

Desert rodents show large quantities of the NSM in the neural lobe and it decreases on dehydration (Legait & Legait 1962); this phenomenon is also noticed in camels (Charnot 1958). Animals with well developed PI counter dehydration better than those with poorly developed PI. It has already been pointed out that the birds which lack a PI seem to survive dehydration for a long time.

In the rat, castration caused a decline of the cell size of PVN (Szentagothai et al. 1968). It has been brought out in the rat (Szentagothai et al. 1968) that endocrine glands influence greatly the hypothalamic nuclei; it is not said whether it is hypo or hyper activity. Ovariectomy had no effect on the arcuate nucleus while oestrogen increased nuclear size of neurons of the arcuate nucleus. Ifft (1964) studying the hypothalamic nuclei after hypophysectomy, ovariectomy, thyroidectomy, adrenalectomy and adrenal medullectomy came to the conclusion that the absence of various hormones does affect the activity of the hypothalamic neurons. Dehydration, thirst, overloading with sodium chloride, heat stress, adrenalectomy and alloxan diabetes deplete NSM (Bern 1966). A neuron may show nuclear enlargement, eccentric displacement of nucleus, enlargement of nucleolus and dissolution of Nissl material. Electrical stimulation of

SON and PVN causes the release of vasopressin and oxytocin (Bern 1967).

Each neurosecretory neuron shows a perikaryon from which a long axon and a few dendrites arise. In the Indian squirrel (Vijayan & Sathyanesan 1971), PVN neuron may show multipolar axons. The dendrites may carry NSM which may enter the blood vessels or a dendrite may throw it into CSF in between the ependymal cells as in the frog, *Varanus* etc. It is also suggested that the dendrites ending underneath the ependyma near the ventricle may register physiological changes in the CSF (Clark & Kennedy 1967). In this connexion, the observation of Bern (1966) is of great significance. According to him, the dendrites of neurons which come in contact with the CSF may act also as a osmoreceptor (there is no intrinsic improbability in assuming a dendrite to be both sensory and secretory); it may act both as a osmoreceptor and a photoreceptor. This neuron will conduct impulses to the PN (carrying an antidiuretic octapeptide) or to the ME (carrying a gonadotropin releaser).

The neurons disclose Nissl substance which is also CAH positive neurofibrillae, considerable RNA as the cell is actively synthesizing protein and a number of CAH positive granules (neuromelanin, lipofuchsin, lysosomes, mitochondria, photoreceptive microtubular system, virus particles) which should not be mistaken for NSM. Bern (1967) also includes under this category of substances which mimic secretory inclusions, lamellar systems, crystalline arrays of virus particles, pigmented globules and heavy glycogen deposits.

The neurosecretory cells receive cholinergic and adrenergic afferent fibres and upon electrical stimulation secrete 'posterior lobe' hormones (Stutinsky 1970).

The intra-axonal movement of neurosecretory granules was first described by

Weiss and Hiscoe (1948); it was about 1—3 mm/day. It has now been found to be even 350—400 mm/day (Bern 1970). Active transport has been found by using radioactive amino acids; radioactive cysteine was administered and there was an active uptake in the magnocellular nuclei. In the hypophysectomised rats, an accumulation of the isotope appeared at the stump of the stalk ten hours later. This proximo-distal flow can be accelerated by different physiological conditions.

Blood-Brain Barrier

While there is a blood-brain barrier in other parts of the hypothalamus, in the external zone of ME large molecules (releasing factors, dopamine, hormones of the anterior pituitary gland) can cross over into the blood vessels as these are permeable (Clementi & Ceccarelli 1970). In the neurohypophysis also, a blood-brain barrier is absent (Bern et al. 1962). Hyyppä et al. (1971) indicate that FSHRH when administered systemically crosses the blood-brain barrier and enters the CSF. Similarly progesterone is found to cross the blood-brain barrier and is metabolised in the brain (Seiko et al. 1969). In order to further establish that CSF acts as a hormonal pathway to convey chemicals into the specialized ependyma cells, David and Anand Kumar (1974) injected *iv* labelled testosterone, progesterone, 17 α -hydroxyprogesterone, mestranol and norethynodrel into monkeys. It was found that all these entered the CSF. 17 α -hydroxyprogesterone, mestranol and norethynodrel concentration was more in the CSF while in the plasma that of testosterone and progesterone was more.

In the cerebral cortex, the blood-brain barrier is very apparent as the neuronal processes are not in direct contact with the basement membrane. Between the

blood and the neuron are noticed: (a) the vessel endothelial cells whose contacts are tightly closed contacts (?), (b) basement membrane, (c) glial processes, and (d) the neuron surface membrane. Any or all of these structures may form the barrier (Gray 1961).

Effects of some Hormones on FSHRH

Recently some experiments have been carried out on the effects of hormones on FSHRH using rat by the Milan School (Motta 1970). The results can be summarised as follows:

1. In the ovariectomised female, the quantity of FSHRH increases more than in control; it is almost double. In the male control, the FSHRH is negligible. The male hypothalamus is different from that of the female for in the latter, there is considerable FSH—RH.
2. Oestradiol decreases the quantity of FSHRH in both male and female castrates.
3. Progesterone depletes FSHRH almost completely in the ovariectomised females, while in castrate males, the FSHRH is quite considerable after progesterone treatment and equals that in control (castrate) which is very large.
4. Testosterone reduces FSHRH to a slight extent in ovariectomised female, while in castrate males, testosterone reduces it to half castrate control level.
5. FSH reduces the content of FSHRH in castrate male rats considerably and also in ovariectomised female rats (Motta et al. 1969).

Based on these findings, the above au-

thors postulate that suppression of feedback signals (long or sex steroids, short or FSH and ultra-short or FSHRH) will activate FSH-FSH-RH system while the opposite will depress it (Motta 1970).

Neuroendocrine Reflex

An examination of the activities of these neurohormones discloses a very interesting interrelationship. They form a circuit by which sensory information is translated from neuronal to hormonal signals and finally into a physiological response and this may be called a neuroendocrine reflex (Frye 1968). The neurohormone may act directly on the target organ; this is the first order and is seen in the action of vasopressin and oxytocin on kidney or mammary glands. The second order is one in which the neurohormone acts upon another endocrine gland (PG) which releases hormones like growth hormone (somatotropin) and prolactin which act on growth and mammary glands. The third order is one in which the neurohormone acts upon an endocrine gland (PG) which produces tropic hormones like TSH, ACTH and GTH (gonadotropic hormones) and these act upon target organs like the thyroid, adrenal and the gonads which in turn may produce hormones acting on their own target organs.

Evolution of the Hypothalamo-Hypophyseal Interrelations

In the primitive condition (Etkin 1962), it may be assumed that a part of the neurosecretion (coming from the neurosecretory nuclei) entered the 3rd ventricle and passed through the ependyma of the infundibular recess into the pial surface adjacent to the PG (figure 55). The release of the neurosecretory hormones into the 3rd ventricle is of common knowledge in vertebrates now. The passage of the neu-

rosecretory material from the 3rd ventricle by the modified fibres of the infundibular recess to the pial surface has been described (Löfgren 1959). The other neurosecretory fibres come to the infundibular recess and end up near the pial surface. Such an ending of fibres has been noticed in cyclostomes (Bargmann 1953). These fibres end up near the pial surface probably releasing the neurosecretion instead of into blood vessels as the latter are scarce in this region and probably also due to a blood-brain barrier. The hypophysis differentiated into two parts, viz., PA and PI. In fishes, the neurosecretory fibres invaded the PI and from such a primitive condition, one could derive the condition in Amphibia where one set of fibres end near blood vessels in the PN and another set passed through the PN and distributed itself in the parenchyma of the PI where blood vessels are sparse. The fibres going to the PI are inhibitory in nature as already indicated. The other line of evolution involves the occurrence

of blood vessels at the region of contact between the infundibular recess and the PA. These capillaries received their neurosecretion from CSF and also from the neurosecretory fibres. This stage, before the appearance of the ME and its portal vessels in Amphibia and Amniota, is visualised in some fish, salamander, anuran larvae and mammalian foetus. It may be pointed out here that the primary plexus in the bird ME is more superficial on the ventral aspect of it, while in Amphibia and Mammalia, it is deeper in the ME.

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EVOLUTION OF HYPOTHALAMO-HYPOPHYSIAL INTERRELATION

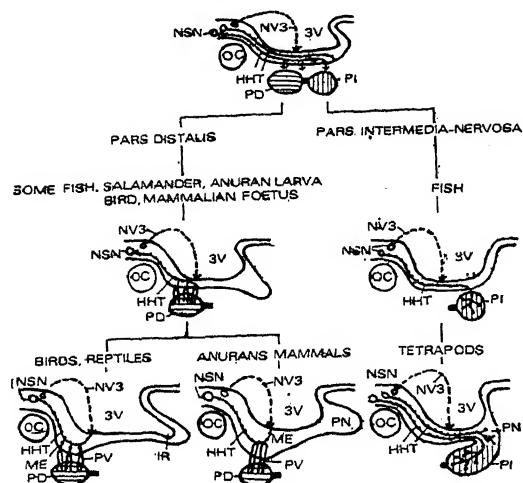


Figure 55 Evolution of the hypothalamo-hypophyseal system (After Etkin 1962, modified)

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List of Abbreviations

| | | | |
|------------------------|--|--------------|--|
| AB, | arterial branch in the pars distalis | AR, | arteria retrochiasmatica |
| ABH, | additional vessel to the primary plexus | ARN, | arcuate nucleus |
| ABI, | anterior branch from the infundibular artery | ART, | the space is an artefact |
| AC, | anterior commissure | AS, | arterial supply to the infundibulum |
| AF⁺, | AF positive fibres to median eminence plus pars nervosa | ASB, | arterial supply to the brain |
| AF⁻, | AF negative fibres from the gonadotropic centre to the median eminence | AVL, | artery to the ventral lobe of the pituitary |
| AHA', | anterior hypothalamic artery | AVME, | afferent vessel to the median eminence |
| AHM, | arteria hypophyseos media | BA, | basilar artery |
| AHYA, | anterior hypophysial artery | BC, | blood capillary |
| AIPP, | infundibular vessels to the primary plexus | BIPP, | branches from the infundibular artery to the primary plexus |
| AM, | amygdala | BM, | basement membrane |
| AME, | anterior median eminence | BNC, | neurosecretory cell bridging supraoptic and paraventricular nuclei |
| AMV, | anterior mesencephalic vessel | BP, | bulbous protrusions |
| ANR, | anterior ramus supplying blood to the brain | BPL, | basal plate |
| APD, | anterior AF positive part of the pars distalis | BV, | blood vessel |
| APN, | artery to the pars nervosa | C, | capillaries |
| APPD? | artery to the posterior part of the pars distalis? | CA, | carotid artery |
| APON, | afferent vessel to the pre-optic nucleus | CAP, | caudal pars distalis |
| APVT, | afferent vessel to the pars ventralis tuberis | CAPV, | portal veins of the caudal pars distalis |
| | | CC, | corpus callosum |

- CCV**, coated caveolae by vesiculation
CER, cerebellum
CHA, commissura habenula
CHI, commissura hippocampus
CMV, coated microvesicles
CP, capillary plexus
CPL, choroid plexus
CPN, capillary plexus in pars nervosa
CPO, commissura posterior
CPP, commissura pali posterior
CPS, capillary 'skeins' originate from the external plexus horizontal shelf and descend to join the external plexus capillaries in the walls of median eminence
CRP, cranial pars distalis
CRPV, portal veins of the cranial part of the pars distalis
CSP, capillary spikes of the posterior tuberalis originate from the external plexus posterior to the infundibular stem and go to the hypothalamus
CV, central vessel

DA, dopamine
DAH, direct amygdaloid-hypothalamic pathway
DB, diagonal band
DCR, dorsal chiasmatic region
DE, dendrite going to ependyma
DMN, dorsomedial nucleus
DPMN, dorsal premammillary nucleus

E, Epinephrine
EC, endothelial cell of blood capillary
EF, foot of ependymal cell with synaptic vesicles
EP, ependymal layer
EPC, ependymal cell
EPHV, encephalo-posthypophyseal portal vessel
EPI, epithalamus
EPL, external plexus
EPP, epiphysis
EPPH, external plexus of the posterior tuber in the infundibular stem going to the hypothalamus
EXO, exocytosis

F, fibre layer in zona interna
F? fibres from unknown source
FAS, fluorescent neurons of the aminergic system
FB, fibroblast
FE, finger-like microvilli
FEN, fenestration in the wall of the capillary
FIR, floor of the 3rd ventricular infundibular recess
FIV, fenestra interventricular
FOR, fornix
FPV, first portal vein
FRH, follicle stimulating hormone releasing hormone cell
FTA, FRH triggering axon

G⁺, gomori positive substance in the ependyma cells

- GL,** glia cell
GLO, glomerulus
GN, gomori negative neuron
GOC, gonadotropic centre
GP, gomori positive neuron
- H,** hypendymal layer
HA, hypophysial artery
HAB, habenula
HC, hypophysial cavity
HG, horizontal groove on the ventral aspect of the brain of some lizards
HHT, hypothalamo-hypophysial tract
HI, hippocampus
HIT, hypothalamo-infundibular tract
HN, habenula
HNA, hypophysio infundibular artery
HNC, higher neural control of magnocellular part of pre-optic nucleus
INCG, higher neural control tract to gonadotropic area
HNT, hypothalamo-neurohypophysial tract
HPA, hypophysiotropic area
HS, hypophysial stem
HV, hypophysial vein
HY, hypendyma
HYA, hypophysial artery
HYA', hypophysial artery going to primary plexus
HYN, hypothalamic nuclei
HYT, hypothalamus
- IA,** islands in adenohypophysis
ICA, internal carotid artery
IHA, inferior hypophysial artery
IN, infundibulum
INA, infundibular artery
INV, injected blood vessel
IR, infundibular recess
IS, infundibular stem
- LI,** lobus inferiorus
LC, microvesicles losing their coat
LG, large granule
LP, lateral pathway
LPD, phloxine positive posterior part of pars distalis
LPV, long portal vein
LPZ, lateral palisade zone
LRH, luteinizing hormone releasing hormone cell; in figure 52; it is LRH bouton
LSV, lateral saccus vasculosus
LT, lamina terminalis
LTA, LRH triggering axon
LV, lateral ventricle
LYS, lysosome degrading the smooth microvesicles of synaptic vesicles
- MB,** mammillary body
ME, median eminence
'ME', median eminence peculiar to *Myxine*
MEC, mesocoel
MEPC, median eminence portal capillary
MES, mesencephalon

| | | | |
|--------------|---|--------------|---|
| MF, | marginal fold | NSG, | neurosecretory granules |
| MFL, | mesencephalic flexure | NSN, | neurosecretory neuron |
| MHT, | mesencephalo-hypothalamic tract | NSPP, | neurosecretion near primary plexus |
| MN, | mammillary nucleus | NSV, | nucleus of saccus vasculosus |
| MP, | medial pathway | NV3, | neurosecretory tract in 3rd ventricle |
| MPN, | magnocellular part of pre-optic nucleus | | |
| MPZ, | median palisade zone | OARN, | outline of arcuate nucleus |
| MSV, | medial saccus vasculosus | OC, | optic chiasma |
| | | OHA, | outline of hypophysiotropic area |
| NC, | neurosecretory cell | ON, | optic nerve |
| NE, | norepinephrine | OTV, | outline of 3d ventricle |
| NGE, | neurosecretory colloidal globule entering the 3d ventricle through the ependyma | OVLt, | organum vasculosum lamina terminalis |
| NGH, | neurosecretory colloidal globule in the hypothalamus | | |
| NGME, | neurosecretory colloidal globule in the median eminence | P, | palisade layer (zona externa) |
| NGT, | neurosecretory colloidal globule in the hypothalamo-neurohypophysial tract | PB, | pineal body |
| NGV, | neurosecretory colloidal globules in the 3d ventricle | PD, | pars distalis |
| NL, | neurosecretory loop | PDOP, | pars distalis nuclei of paraventricular organ |
| NID, | nucleus infundibularis dorsalis | PE, | pinocytosis |
| NIL, | neurointermediate lobe | PFMB, | post-hypothalamic nucleus with axons going to mesencephalon |
| NLT, | nucleus lateralis tuberis | PFN, | posterior parafascicular nucleus |
| NS, | neurosecretion (peptidergic) | PHA, | posterior hypothalamic artery |
| NSA, | neurosecretory axons | PHMB, | posterior hypothalamic fibres to mesencephalon |
| NSC, | neurosecretory cell in the median eminence and mesencephalon | PHN, | posterior hypothalamic neurosecretory nucleus plus fibres to mesencephalon |
| NSE, | neurosecretory ending in the infundibulum | PHNP, | axons from the posterior hypothalamic neurosecretory nucleus to neurohypophysis |
| NSEV, | neurosecretion passing between ependyma cells into the 3d ventricle | PHT, | preoptico-hypophysial tract |

| | | | |
|---------------|---|--------------|--|
| PI, | pars intermedia | PRO, | preoptic recess organ |
| PIH? | prolactin inhibiting hormone? | PRT, | preoptic recess organ tract |
| PIN, | plexus in pars intermedia | PS, | perivascular space |
| PIR, | piriform cortex | PT, | pars tuberalis |
| PLPD, | phloxine positive part of pars distalis | PV, | portal vein |
| PM, | preammillary nucleus | PVN, | paraventricular nucleus |
| PME, | posterior median eminence | PVNI, | portal vein to neurointer- mediate lobe |
| PMG, | posterior mesenteric group of vessels | PVO, | paraventricular organ |
| PN, | pars nervosa | PVOT, | paraventricular organ hypo- physial tract |
| PNDC, | pars nervosa distal contact | PVP, | portal vessels on the post- erior face coursing towards the hypothalamus |
| PO, | pons | PVT, | portal vein in the pars terminalis |
| POA, | preoptic area | | |
| POC, | postoptic commissure | R, | reticular layer (zona externa) |
| POHB, | preoptic tract to hind-brain | RH, | hypophysial recess |
| POHTL, | preoptic fibres entering neurohypophysis laterally | RME, | rostral median eminence |
| POIT, | preoptico-infundibular tract | RPD, | rostral pars distalis |
| PON, | preoptic nucleus | | |
| PON', | preoptic nucleus anterior | SC, | secretory cells |
| PONR, | preoptic nucleus axon to rostral pars distalis | SCH, | suprachiasmatic nucleus |
| POPN, | nucleus post-opticus | SCL, | simple capillary loop |
| POR, | preoptic recess | SCO, | subcommissural organ |
| PP, | portal primary plexus | SE, | special ependymal cell |
| PPD, | proximal pars distalis | SEC, | septum complex |
| PPGF, | pars proximalis showing green fluorescence | SEGP, | special ependyma gomori positive cell |
| PPH, | paraphysis | SEL, | subependymal layer |
| PPN, | parvocellular part of preoptic nucleus | SEPV, | second portal vein |
| PPYF, | pars proximalis showing yellow fluorescence | SG, | small granule |
| PRI, | posterior recess of the infundibulum | SHA, | superior hypophysial artery |
| | | SMT, | sulcus medialis thalami |
| | | SMV, | smooth microvesicles or synaptic vesicles |

- SON,** supraoptic nucleus
SPD, sinuses in pars distalis
SPS, secondary portal plexus or sinus
SPV, short portal vessel
ST, stalk of the pituitary gland
STR, stria terminalis
SV, saccus vasculosus
SY, synapsis
SYV, synaptic vesicle

T, double neurosecretory tract from the median eminence
TC, tuber cinerium
TD, dorsal thalamus
TE, telencephalon
TH, thalamus
TIT, tubero-infundibular tract
TIT, dopamine tract
TOL, lateral olfactory tract
TPV, third portal vein
TT, tuberal tract
TV, ventral thalamus

TVS, tufted vessels arising from the external plexus and joining the central vessel

UN, unmyelinated fibre

V, vein to systemic circulation
VDI, vessel from pars distalis to pars intermedia
VL, ventral lobe
VLA, vena lobi anteriores
VMI, vessel between median eminence and pars intermedia
VMN, ventromedial nucleus
VN, vascular network
VVL, vein to ventral lobe
VPPH, vessel linking primary plexus and the hypothalamus

ZT, zona tuberalis
3V, 3rd ventricle

1, 2, 3, 4, 5 branches forming preoptico-neurohypophysial tract in *Anguilla*

Errata

| Page | Col. | Line | Instead of | Read |
|------|--------|---------------|---------------------------------------|---|
| 1 | Title | | Vertebrate Neurosecretion—Review | Vertebrate Neurosecretion—A Review |
| 1 | 2 | 5 | to the hypothalamus to the hypophysis | to the hypothalamus from the hypophysis |
| | 2 | 5 | delete 'the neuron'. | |
| 1 | 2 | 22 | (Gorbman & Bern) 1962) | (Gorbman & Bern 1962). |
| 4 | 2 | 9 | cours ing | cours-ing |
| | 2 | 18 | th | the |
| 5 | 1 | 3 | he | the |
| | | (from bottom) | | |
| 11 | 2 | 1 | he blood | the blood |
| 15 | 1 | 16 | (Stomtas) | (Stomias) |
| 15 | 1 | 34 | 800–250Å | 800–2500Å |
| 15 | 2 | 6 | exterpatation | extirpation |
| 16 | 2 | 4 | 100 mm | 100 nm |
| | | 5 | meter as Gomori and AF positive | meter and is Gomori and AF positive |
| 22 | 1 | 19 | Uracotyphlus | Uraeotyphlus |
| 27 | 1 | 12 | tadpoles | tadpole's |
| 28 | 1 | 28 | peptdergic | peptidergic |
| 29 | Fig 32 | 2 | withneurosecretory | with neurosecretory |
| 30 | 2 | 18 | (700 Å fibres | (700 Å) fibres |
| | | 22 | (1000–3000 Å fibres | (1000–3000 Å) fibres |
| 31 | 2 | last line | Bargmann et al. 1950) | (Bargmann et al. 1950) |
| 35 | 1 | 30 | Assenmocher | Assenmacher |

| Page | Col. | Line | Instead of | Read |
|-------------|-------------|-------------|---|--|
| 35 | 2 | 14 | sion, Oksche 1971), p. 907) | sion, Oksche 1971, p. 907) |
| | 2 | 36 | m cro-villi | microvilli |
| | 2 | 37 | hel pin | help in |
| 36 | 2 | 19 | Hill and Hender on | Hill and Henderson |
| | | 39 | (Kobayashi et al. 19,0), | (Kobayashi et al. 1970) |
| 38 | 1 | 23 | (Flerk 1970) | (FlerkÓ 1970) |
| 40 | 1 | 19 | (Averill & Kenredy 1967); | (Averill & Kennedy 1967); |
| 41 | 1 | 42 | (Thyroliberin Mark 1976) | (Thyroliberin Marks 1976) |
| 44 | 2 | 4 | <i>Polypeptide Ho,mones</i> (from bottom) | <i>Polypetide Hormones</i> |
| 46 | 2 | 19 | started | stated |
| | 2 | 2 | apparatus' | apparatus |
| | | | (from bottom) | |
| 50 | 1 | 24 | in | |
| 51 | 2 | 2 | Vijiyan and McCann (1977, in press) | Vijiyan and McCann (1978) |
| 53 | 2 | 19 | Disconnected | disconnected |
| 59 | 1 | 25 | al | al- |
| | | 33 | ovariectomisec | ovariectomised |
| 62 | 1 | 24 | Anand Kumar T A | Anand Kumar T C |
| | 2 | 7 | guide | crude |
| 63 | 1 | 21 | Bern H A and Knowler F G W | Bern H A and Knowles F G W |
| | 1 | 12-17 | Shift Journal. vol. page <i>after</i> the title in the | reference Bower, Hadley and Hruby 1971 |
| | 1 | 8 | Zour Feinstrutur | Zur Feinstruktur |
| | | | (from bottom) | |
| | 1 | | <i>Supply</i> | <i>Supple.</i> |
| | | | (from bottom) | |
| 63 | 2 | | "Campbell H J 1966..." | Campbell H J 1966 The development of the primary plexus in the median eminence of the rabbit; <i>J. Anat. Lond.</i> 100 381-387 |
| 64 | | 5-8 | Shift matter "from the pars.....190-209" to Col. 2 to come under Van Dyke, Chow, Greep and Rothen 1941 (p. 64, column 2 after line 8 from bottom) | |

| <i>Page</i> | <i>Col.</i> | <i>Line</i> | <i>Instead of</i> | <i>Read</i> |
|-------------|-------------|---------------------|--|-----------------------------|
| 64 | 2 | 7 (from bottom) | Rotham | Rothen |
| 64 | 1 | 1 | Cecarelli | Ceccarelli |
| 65 | 2 | 11 | delete Frieden E | |
| 65 | 2 | 32 | Name of 3rd author not clear | (Löfström) |
| | 2 | 44 | Name of 4th author not clear | (Löfstrom) |
| | 2 | 50 | Name of 2nd author not clear | (Löfstrom) |
| 66 | 2 | 23 | School | Schaal |
| 68 | 2 | 16 | nervsa | nervosa |
| 70 | 2 | 10 | secrtion | secretion |
| | 2 | 7 (from bottom) | Sraschini | Fraschini |
| | 2 | 5 (from bottom) | delete 'and' before McKanee and add 'and Parsons J H after McKennee C T' | |
| 71 | 1 | 6 | delete (in <i>Biochem. Biophys. Res. Comm.</i>) | |
| 72 | 2 | 17 (from bottom) | Lovine | bovine |
| | 2 | 13 (from bottom) | 1968? | 1968 |
| | 2 | 7 (from bottom) | Zwischenhirn | Zwischenhirn |
| 73 | 2 | 5 | Steward A D 1971 | Stewart A D 1968 |
| | 2 | 7 | <i>Musmusculus</i> | <i>Mus musculus</i> |
| | 2 | 24 | Taleisnik S and Tomats ME | Taleisnick S and Tomatis ME |
| | 2 | 13 (from bottom) | Tórök | Török |
| 77 | 1 | 19 | hypophysio infundibular | hypophysio-infundibular |
| 78 | 2 | 9 (from bottom) | fibreo | fibres |
| 79 | 2 | 9 (from bottom) | gomori | Gomori |



Professor L S Ramaswami is a distinguished and versatile zoologist of the country. He received his D.Sc. degree from the University of Madras and taught at the Universities of Mysore and Rajasthan. His early researches include the embryology of bats and the cranial osteology of fishes, amphibia and reptilia, notably structural peculiarities of the skull and the Weberian ossicles of the family Cyprinidae in relation to phylogeny, induction of spawning in catfish using pituitary hormones. He started a fish physiology laboratory at Bangalore in 1954, using the air-breathing catfish *Heteropneustes fossilis* as the experimental system and also extended his interests to the comparative endocrinology of the frogs, particularly the skipper frog, *Rana cyanophlyctis*.

He was responsible for building a modern endocrinology laboratory with an electron microscope and a well-equipped animal house at the University of Rajasthan, Jaipur. His group produced excellent work on the reproductive biology of the langur monkey and the pusillanimous *Loris*.

Professor Ramaswami's works drew the scientific attention of stalwarts abroad, and he subsequently established close research collaboration with some of them.

Professor Ramaswami has published over 100 technical papers and his works are extensively cited in reviews and books. He was invited to several important international symposia in India and abroad to present technical papers.

He was elected a Fellow of the Indian National Science Academy in 1957 and was honoured with the coveted Sunder Lal Hora Medal (1975), in recognition of his outstanding contributions. He presided over the Zoology Section of the Indian Science Congress at Cuttack in 1962.

For Professor Ramaswami age is only an event for the calendar. Embers of his scientific enquiry keep glowing unquenched.